A Study of the Relationship of Abnormal Reward Processing and Dopamine Signalling in Adults with High Functioning Autistic Spectrum Disorder

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Abstract

Introduction: Autistic spectrum disorder (ASD) is a neurodevelopmental condition which is defined by language and communication difficulties, impairment in social skills and restrictive and repetitive patterns of behaviour. Functional deficits have often been observed in individuals with autistic spectrum disorder, which could be partially due to an early failure of the amygdala. Motor activity, attentional skills, social behaviour and perception of the outside world are all implicated in autism and are all also modulated by the neurotransmitter dopamine. Past evidence has suggested that dopamine neuron activation aids a person on learning to identify the association of particular stimuli with reward.

Aims*:* The main objective of this preliminary study is to examine the neural activation during the visualisation of grouped images from the IAPS library in normal participants and to observe if the activation follows a pattern in the neural reward circuitary. ***Methodology****:* Participants underwent functional magnetic resonance imaging (fMRI), during which they performed four tasks to test their reward processing; a visual paradigm, in which the participants saw a display of images with differing emotional weightings, a gambling paradigm, a soft touch paradigm and a materials paradigm. The visual paradigm fMRI data was analysed using Brain Voyager version 1.3. ***Results****:* The limbic lobe, anterior cingulate gyrus and the frontal lobe were the regions of the brain most activated for all of the visual conditions in the control participants. The ASD participant activated more neural regions, especially the limbic regions, than the control participant in the Case vs. Control comparison. ***Discussion****:* Common areas of activation in the control group were the anterior cingulate gyrus, the limbic lobe and the medial frontal gyrus. These areas all modulate facial and image recognition, emotional processes and the evaluation of reward. The ASD participant demonstrated more activation in limbic regions of the brain than the control participant. These results support the idea that individuals with ASD have difficulties in controlling their arousal state and often have high levels of arousal. This can have implications on the processing of rewarding and non rewarding stimuli in people with ASD.

Nomenclature

ASD	Autistic Spectrum Disorder
MRI	Magnetic Resonance Imaging
fMRI	Functional Magnetic Resonance Imaging
IAPS	International Affective Picture System
ABA	Applied Behavioural Analysis
PET	Positron Emission Tomography

Contents

1.1i Autistic spectrum disorder: definitions, diagnosis and management.

A person's social, academic and emotional life depends on adequate communication with the world around them. Each individual must exchange some sort of contact with people that they meet in order to sufficiently move forward in their lives. Autistic spectrum disorder (ASD) is a neuro-developmental condition which is defined by language and communication difficulties, impairment in social skills and restrictive and repetitive patterns of behaviour. The term 'spectrum' is used due to the variation in the severity and pattern of symptoms from person to person. It was not until the early 1940's that a label was introduced for this disorder that has been found to affect many children and adults.

History:

In 1943 Dr. Leo Kanner assessed a group of 11 children and introduced the name of *early infantile autism*; whilst at the same time Dr. Hans Asperger, an Austrian paediatrician, described a milder form of the disorder that became known as Asperger's syndrome (1). These two disorders are now listed in the Diagnostic and Statistical Manual of Mental Disorders DSM-IV-TR (2) as two of the five pervasive developmental disorders (PDD), referred to today as autistic spectrum disorders (ASD). These disorders are all characterized by delays in the development of multiple basic functions including socialization and communication, ranging from the severe form, autistic disorder, to a milder form, Asperger's syndrome. If a child has symptoms of autistic disorder or Asperger's syndrome, but does not meet the full criteria of either of these, their diagnosis is called pervasive development disorder not otherwise specified (PDD-NOS). Other conditions included in the autistic spectrum are Rett's syndrome and childhood disintegrative disorder. Rett's syndrome is a progressive neurodevelopmental disorder affecting 1 in 10000 females (3). Usually patients develop normally until approximately six to eighteen months of age, following which the child regresses, losing their acquired skills such as speech, motor skills and purposeful hand movements (4). Girls who suffer from Rett's syndrome will then frequently develop microcephaly, seizures, ataxia and autism (5). Although the symptoms of this condition are severe, most individuals with Rett's

syndrome live well into their middle ages and beyond (6). Childhood disintegrative disorder is a condition which occurs in three to four year olds who develop normally until approximately two years of age, following which they suddenly deteriorate in their intellectual, social and motor functioning. It has been noted that children who suffer from this rare syndrome demonstrate autistic like tendencies such as impairments in non verbal behaviour and a loss of social and language skills (7). Over the years various terms such as disintegrative disorder, disintegrative psychosis and Heller's syndrome have been employed to describe this condition (8). There is no known etiology for this disorder and the developmental delay continues throughout the individual's adult life (9).

***Epidemiology*:**

ASD affects approximately 1 in 100 children in the UK, as estimated by the National Autistic Society (10) **,** with the risk 3-4 times higher in males than in females. The prevalence of autism has been calculated in many areas and there have been differences in the exact prevalence of autism due to the varied methods of diagnosis between health authorities and individual health professionals (11). In recent years the estimated prevalence of ASD has ranged between 12.2 to 67.4 cases per 10,000 population depending on the location and the research undertaken (12). There has been an increase in the number of reported cases of ASD in the last two decades (13). It is argued that this increase could be due to a number of factors. The body of knowledge about the condition is rapidly expanding along with an escalation in public awareness. The increased knowledge and the development of the concept of the wide autistic spectrum allows for improved case recognition and the possibility of a genuine increase in numbers (14).

A number of environmental causes for a rise in incidence have been discussed by researchers (14), including the question as to whether the triple vaccine for measles, mumps and rubella could have been a potential cause for a few cases of childhood autism. However, this concern was not confirmed by scientific backing and was denied after various investigations (14)(15). There is strong evidence to suggest that genetic factors have a significant role to play in the aetiology of ASD. It has been shown that the suggested phenotype for autism is more likely to appear in a monozygotic twin of an individual diagnosed with ASD, in comparison to a

dizygotic twin (16)(17). A review by Rutter showed data indicating that siblings of individuals with autism were more likely to be affected by autism compared to the general population and subsequent studies confirmed those results(18) (19)(20). Another argument which favours the genetic basis of autism is the broad phenotype of the disorder, which includes a range of symptoms from social communication difficulties shown in Asperger's syndrome to mild autistic features such as highly focused interests and activities (21). First degree relatives of individuals with ASD tend to have an increased risk of displaying this broader phenotype.

The improved recognition of ASD traits at a younger age may have contributed to the increased number of diagnosed cases of ASD over recent years (22). There was little interest in ASD before the 1960's. Parent and support groups began to form in USA and UK, which encouraged medical and educational professionals to become more informed about the symptoms and methods of management for the disorder (14). Following the initial surge of groups forming and their push to have ASD publicised in the media, awareness of the wider view of ASD began to expand (14). Broadening of the criteria used to assess and diagnose ASD has contributed to health professionals feeling more prepared to diagnose individuals with the disorder. A characteristic of ASD is the increased prevalence in males, with autistic individuals showing a sex ratio of 4:1 (male: female) and individuals with Asperger's syndrome having a sex ratio of 9:1 (male: female) (23)(21). Whether the apparent increase in prevalence of ASD is due to a true increase in the condition or an increase in knowledge and recognition of the disorder (24,25), the increase in prevalence clearly demonstrates the need for a greater understanding of the condition.

***Terminology and diagnosis*:**

The current diagnostic criteria for autistic disorder, from the Diagnostic and statistical manual of mental disorders: DSM IV, contain three core domains and for a diagnosis of autism to be made, an individual must exhibit at least six symptoms falling within the three core domains (socialisation, communication and restricted behaviours, interests and activities) (26).

A. Qualitative impairment in social interaction, with at least two of the following:

- impairment in the use of non-verbal behaviours
- inability to develop peer relationships
- lack of ability to seek sharing enjoyment
- interests or achievements and lack of capability of social or emotional reciprocity.

B. Qualitative impairment in communication, with at least one of the following:

- delay or lack of spoken language
- inability to begin or sustain a conversation
- repetitive language or lack of make believe play.

C. Restricted repetitive and stereotyped patterns of behaviour, interests and activities, with at least one of the following:

- a preoccupation with a stereotyped behaviour, interest or activity
- a rigid obedience in following specific routines or rituals
- repetitive motor mannerisms
- preoccupation with parts of an object.

To complete the diagnosis of autism, an individual must demonstrate delays in one or more of the following areas, with the onset before the age of 3 years:

1. Social interaction.
2. Language as used in social communication.
3. Symbolic or imaginative play.

Asperger's syndrome is characterised by impairments in social skills, such as difficulties in conversation and play and an inability to make appropriate eye contact or to recognise facial expressions (13), but without difficulties in language fluency or academic abilities. The diagnostic criteria for Asperger's syndrome are similar to that for autistic syndrome, with two of the core domains (A and C) used to diagnose autistic syndrome, also seen in Asperger's syndrome (26)(27). There also exist further categories for the diagnosis of Asperger's syndrome that differentiate it from

autistic syndrome, as stated in the Diagnostic and statistical manual of mental disorders: DSM IV:

1. The disturbance causes clinically significant impairments in social, occupational, or other important areas of functioning.
2. There is no clinically significant general delay in language.
3. There is no clinically significant delay in cognitive development or in the development of age-appropriate self help skills, adaptive behaviour (other than in social interaction) and curiosity about the environment in childhood.
4. Criteria are not met for another specific Pervasive Developmental Disorder or Schizophrenia.

All people with ASD demonstrate difficulties with social interaction and verbal and non-verbal communication. Additionally, repetitive, bordering on obsessive, behaviours and interests are common in children and adults with ASD. As described in Kanner's work on autism, the group of children that he studied seemed to appear aloof and lack concern for other people (1), whereas Asperger's published work depicting children who showed similar symptoms to those from Kanner's work. The only difference between the groups of children was that Asperger's group of children demonstrated higher verbal and cognitive skills.

ASD can very often be recognised in children as young as 1 year by their parents picking up warning signs that their child is not behaving in the way that they would expect. Their child may seem to be unresponsive to playful behaviour and may be quiet and withdrawn in comparison to children of a similar age.
The surfacing of these warning signs should indicate the need for the child to be evaluated by a professional who specialises in ASD.

The manifestations of autism range from people with severe impairments who may have severe learning difficulties and undertake repetitive actions, to high functioning individuals who have focused interests and methods of communicating

and can, to a certain extent, cope in many social circumstances. Autism is often divided into low, medium or high functioning autism, related to the individual's intellectual abilities (28), although this method of categorising people with autism is not used in all centres that diagnose the condition.

***Differences between Asperger's syndrome and autism*:**

The pervasive developmental disorders (Asperger's syndrome, disintegrative disorder and atypical autism) show phenotypic overlaps with core autism, so autistic spectrum is increasingly being used to demonstrate the continuum between the conditions (21). However, it is unknown whether autism consists of a variable single syndrome or a collection of individual syndromes that share similar features.
Many researchers argue that there are differences in the early presentation of children with high functioning autism and Asperger's syndrome, as on the whole Asperger's syndrome presents without learning difficulties, although it can co-exist with learning problems (26,29,30). In 1994 the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), described the diagnostic criteria for Asperger's syndrome. The authors of the manual, revised in 2000 DSM-IV-TR (2), stated their opinions that Asperger's syndrome could be differentiated from autism by examination of the child's early development. Asperger's syndrome differs from autism in that individuals show no general delay in language or cognitive development (30). Certain characteristics such as early language development and cognitive skills are delayed in children with autism; however these features are not significantly slower in children who have Asperger's syndrome (31). It is noted that many people with autism isolate themselves and make no attempts to socialize with others in their peer groups, whereas individuals with Asperger's syndrome by and large make an effort to interact with other people, even if their manner of socializing may be repetitive and unusual to others.
The term of Autistic spectrum disorder is used to group together individuals who diagnostically suffer from different conditions, however clinically experience similar symptoms and deficits in certain areas of their functioning albeit in various severities.

“It must be remembered that autism is diagnosed by the existence of the full triad of impairments and the particular manifestation of the triad will vary among individuals. There are no behaviours per se that by their presence or absence indicate autistic spectrum disorders; it is the overall pattern and underlying difficulties that define autism” (32).

Functional Deficits:

There are a number of functional brain deficits that have been identified in people suffering from ASD (33-36). These functional deficits are well defined and consist of deficits in executive function (33), verbal comprehension, vocabulary and comprehension (35). Executive dysfunction is another broad category that has been observed in not only developing children who have ASD, but also adults (37). Executive function refers to a set of cognitive abilities that control other behaviours and are essential for goal directed, voluntary decision making. Some clinical features of high functioning individuals with ASD such as obsessional behaviour and repetitive actions have been related to executive functional deficits (38).

Tasks measuring drives for central coherence (39) and theory of mind abilities, which relates to the ability to attribute mental states to others, are related to abnormalities in the frontal cortex and basal ganglia structures (40). One of the most powerful theories regarding the aetiology of ASD is the theory of mind deficit account, which hypothesises that the social difficulties apparent in ASD are due to a lack of understanding of feelings, thoughts and intentions in themselves and other people (22,41). This social cognitive approach to looking at autism is employed by Baron Cohen et al (42), who consider that autistic individuals who lack a theory of mind do not understand that people have different thoughts in their head regarding the world around them. The implications for the social interactions of these individuals include problems in considering other people's feelings and an inability to realise that people have different levels of knowledge compared to them.

The characteristic triad of communication, social and imagination dysfunctions have been successfully explained by the hypothesis of individuals with ASD lacking a theory of mind (43,44). However, the non-social impairments shown by people with ASD cannot be explained by this theory. A further theory exists which states that the behaviours and actions of people with ASD are due to the lack of central coherence, which simply means that an individual is unable to incorporate their senses and their behaviour into reasonable actions (45). This theory was developed by Frith (1989) (46) and further expanded by Frith and Happe (1994) (47).

These non-social features consist of restricted interests, obsessive actions to continually have the same environment, restricted areas of ability, an excellent memory and perceptual abnormalities (hypo/hypersensitive to sound, touch, vision, taste and hearing) (44). It is suggested that these features of ASD may be due to a failure to process incoming stimuli in context (44).

The sensory perceptual problems that are commonly reported in ASD can severely affect an individual's daily quality of life (48). These sensory abnormalities can manifest as extreme hypersensitivity to everyday sensory stimulation, causing intolerance to particular situations or sensations, such as loud noises, crowds or tags on clothing. On the other hand, individuals with ASD may be hyposensitive and have an underdeveloped response to the same stimulation types. A hyposensitive individual may use abnormal means to experience sensory input, such as head banging, or bringing everything into close visual range. Dysfunction in tactile perception in ASD can also be seen in withdrawal from social touch, refusal to eat certain textures of food, discomfort in wearing certain materials of clothing, or the use of fingertips rather than the whole hand when manipulating objects (49).

***Management*:**

The three core difficulties that characterize autistic disorders, social interaction, communication and restrictive or repetitive behaviours and interests, manifest in varying degrees of intellectual ability, language skills and behavioural severity in different individuals. ASD covers similar disorders that share a broad behavioural phenotype, that seem to follow the same common pathway of atypical neurodevelopment (31). Health professionals have an important role in recognizing symptoms of ASD and developing strategic management programmes for each individual diagnosed with ASD. The main goals in the management of individuals with ASD is to encourage independent functioning, to promote the development of socialization, reducing maladaptive behaviours, to improve their quality of life and to educate and support each person and their family (50). ASD is generally not termed as a 'curable' condition; therefore management is aimed at long term therapies. Most children with ASD are highly likely to remain in the spectrum as adults and may continue to experience problems in independent functioning, social circumstances,

employment and mental health (50)(51,52). Therefore, it is important for the individual to minimize the core features of the condition and to maximize their independent function and their quality of life through educational development and learning (50). The key management strategies include the following:

- **Educational interventions**:
    1. Behavioural strategies and therapy to develop speech and language are the cornerstones of management of ASD. Not only do these interventions tackle communication and social skills, but daily-living skills, play and leisure skills and academic achievement are also addressed (53). The development of all of these skills that an individual with ASD lacks, allows them to progress in these areas of their lives. In recent years, research looking into management for ASD has focused on young children with ASD, due to the evidence that earlier intervention can significantly improve the future outcome (53). It has been demonstrated that when interventions of this kind are provided from an early age, the effectiveness of therapies are noted in the large number of children benefiting from such therapies (53). Behavioural management therapies focus on reinforcing wanted behaviours and reducing unwanted behaviours.
    2. Areas of education and support that are thought to be beneficial for children, who have ASD, are a curriculum that encourages interaction with people, a predictable routine for the child to feel at ease, a calming approach to dealing with problem behaviour and definite family involvement (54). Various specific strategies have been created to be used in educational programmes for children with ASD. Applied Behaviour Analysis (ABA) uses principals from experimental psychology to create interventions that change unwanted behaviours (50). ABA is used to create and maintain adaptable behaviours, reduce unwanted behaviours, teach new skills and provide various environments for continuous improvements in behaviour in various situations (55). Structured teaching methods such as the TEACCH method, place an emphasis on the organisation of the physical environment, predictable timetable for activities and flexible but structured activity systems (56).

3. A very important factor of management for a child with ASD is parental support and education (57). The provision of support groups can allow parents and carers of people with ASD to have contact with individuals who deal with the same trials and tribulations, who may be able to offer supportive advice (53,54).

- **Therapy**

  The forms of therapy which have a significant impact on an individual's improvement are:

  1. Speech and language therapy (as people with ASD usually have deficits in social communication) (50).
  2. Social skills instruction (targeting the initiation of social behaviour, minimising stereotyped persistent behaviours and teaching social skills) (58).
  3. Occupational therapy (to promote the development of self care and academic skills) (50).

- **Medical management**

  1. Autism therapies are designed to treat the symptoms of autism rather than treat the condition itself. The core problems of autism can be complicated by behavioural problems such as aggression, temper outbursts and irritability (59). Children with autistic spectrum disorder also often suffer from co-existing hyperactivity and lability of mood (60). When behavior therapy and environmental changes do not improve the situation, drug treatment may be considered. Antipsychotic drugs are the mainstay of drug therapy for behavioural problems of children and adolescents with ASD.

     Haloperidol is one of the most widely studied antipsychotic drugs used to reduce symptoms in children and adolescents with ASD (60-62). Safety concerns exist regarding the risk of drug related dyskinesias with the usage of haloperidol, meaning that when prescribed haloperidol must be monitored carefully (62,63).
  2. Neurobiological research has associated disruption in the dopamine and serotonin systems to the pathology of autism (64-66). Serotonin2A-

dopamine D2 antagonist risperidone, an atypical antipsychotic, has been studied extensively and has been deemed to be safe and efficacious at reducing the disruptive symptoms observed in autism (67-69). Risperidone must also be monitored when prescribed for behavioural symptoms in autistic children and adolescents due to the risk of drug induced weight gain and metabolic disturbances (62).

3. Selective serotonin reuptake inhibitors (SSRI's) have been utilised to treat the behavioural symptoms and functional impairments of autism in children and adults (70). Studies have been carried out which suggest effectiveness of fluvoxamine (in children and adults) and fluoxetine (in children); however, there is an increased awareness of the agitation risk in autistic individuals who have been prescribed an SSRI (71,72). There are questions regarding the tolerability and suitable dosing of SSRIs in children and therefore these drugs are monitored vigilantly when prescribed to children (71).
4. General medical management of children and adults with ASD consists of the same health advice and disease prevention that people without ASD also require. However in addition, they may require extra health care needs related to underlying conditions that they may suffer from such as fragile X syndrome or tuberous sclerosis and other health conditions such as epilepsy (50,53). The range for the risk of children with ASD developing epilepsy has varied between 10-30% (73). The prevalence of epilepsy in children with autistic spectrum disorder has varied between various studies, due to the differences in the guidelines used for diagnosis.
5. Many members of a multi-disciplinary team have a role in caring for an individual with ASD. The theory of mind deficits, described in the functional deficits section (page 14), can be recognised by members of the professional team, from signs such as difficulties interacting with others, a lack of understanding of others intentions or feelings and difficulties in social situations. Social workers, care workers and teachers can play a vital role in an individual's development. By finding methods of teaching an individual with ASD and helping them progress in their learning, professionals may be able to find ways of improving the social skills many people with ASD have difficulty with. Health care

professionals can also play a part in helping individuals with ASD in improving their social skills by identifying the signs and by informing the appropriate specialist, such as a psychologist, who can aid each individual in their development.

1.1ii Brain development and ASD

The functional deficits often observed in individuals with ASD have been analysed in a recent study and it has been reasoned that these deficits may be partially due to an early failure of a particular cerebral structure called the amygdala (74). Functional magnetic resonance imaging studies have established that the amygdala shows increased activation when social intelligence is being performed (75). Social intelligence is considered as an individual's ability to interpret another person's behaviour in terms of their mental state, an ability to interact in close social groups and in close relationships, the capability to empathise with another's mental state and to be able to predict how another person feels and how they are going to behave (76). It has been proposed that the amygdala plays a role in the neural network that controls social behaviour (77). The overall volume of the amygdala is significantly reduced and in post mortem studies shows neuropathology in autistic individual's brains in comparison to their age and gender matched controls (78)(79). The malfunction of the amygdala has a large influence on a particular area of the brain involved in visual-social perception such as the fusiform (face area) of the ventral temporal lobe. Neuroimaging work has regularly indicated the large involvement of the fusiform gyrus and the amygdala in the processing of emotion and the medial prefrontal and frontal cortex in tasks involving theory of mind (80). A consistent finding in the neurobiology of autism is the reduced activity of the fusiform gyrus and other cortical areas of the brain which assist with face recognition and visual perception in people with autism (81,82).

The amygdala theory of autism was proposed by Baron Cohen et al after reviewing the evidence of a social function of the amygdala (83). The fMRI study involved autistic and non-autistic participants judging from a person's eyes how they may be feeling, illustrating that the amygdala showed activation whilst non autistic participants were making mental inferences about people's eyes. However, participants with autism did not demonstrate as much amygdala activity as the control participants during the task. The amygdala is therefore proposed to be one of the neural areas that are abnormal in autism.

Recent imaging studies carried out on autistic populations have demonstrated that autistic individuals have a significantly smaller cerebellum in comparison to a

control group comparison (84,85), which is consistent with the results of previous studies showing cerebellar hypoplasia in autistic individuals (86,87). Cerebellar abnormalities, such as hypoplasia and hyperplasia of the posterior vermis and hemispheres, in autistic individuals have been reported in past studies. However these are not only seen in autism but also in neurogenetic syndromes and children with leukaemia who have had radiation to the brain.

Theories have been developed from work showing altered c-fibre functioning in adults with ASD (88,89). The main class of c-fibres are in the skin and they react to painful stimuli. C-tactile fibres are responsible for the pleasurable feeling that touch produces on hairy skin and track directly back to the orbitofrontal cortex and amygdala. It is a possibility that the altered sensory input from these fibres is due to an impairment in normal amygdala development from early childhood. This altered c-tactile fibre function goes hand in hand with the sensitivity deficits observed in individuals with ASD such as the hyper/hyposensitive response to sensory stimuli (90). Some people with autism may be unable to tolerate a well meaning pat on the back, whereas others may engage in self injury without seeming to feel pain. A class of unmyelinated tactile mechanoreceptors that have recently been identified in humans, known as CT afferents, are unmyelinated c fibres that respond to slow, light, stroking touch stimuli (91). These fibres are thought to be only distributed in hairy skin. With their preferred response as pleasant stroking touch, this class of fibres compose a social touch system, which could play a role in the hyper/hyposensitivity associated in autism (91-93).

The failure of development in key brain structures can also be interpreted as a failure in learning, as the common deficits in ASD are in functional areas where learning is vital to normal development. Recent studies regarding the neurobiology of autism support this hypothesis by developing the theories that since the joining of neurons is the neurobiological trigger behind learning, cortical connectivity must be deficient in ASD (94-97).

1.1iii Neurochemistry in ASD

Central to explaining the deficits found in ASD, is the exploration of possible neurochemical mechanisms that may underlie the abnormal brain functioning. Whilst the role of serotonin and other neurochemicals has been explored, there is little work on the role of dopamine in these disorders. This is an important short fall as the functional deficits seen in ASD are in brain areas where dopamine is the predominant neurochemical. Researchers who have studied the neurochemistry of ASD have identified chemical changes that have been found in individuals with ASD (73).

Serotonin

Autistic syndromes have a strong genetic component (98) and a category of genes that have received much attention is the group that encodes proteins necessary for brain serotonin metabolism and neurotransmission (99,100). This interest was due to early findings of increased serotonin in autistic individuals (101)(102) and this topic has been further researched in current studies (66)(65). A number of studies have observed hyperserotonaemia in a third of autistic individuals and their first degree relatives, which suggests that elevated blood serotonin levels could be a marker for genetic susceptibility of autistic spectrum disorders (99,103). However, the difficulty with measuring the levels of serotonin or, for that matter dopamine, in the blood relates to the fact that both of these neurotransmitters cannot cross the blood-brain barrier. Accurate measurements of the concentrations of serotonin and dopamine in the brain consequently cannot be quantified by measuring the amount in the blood.

Further evidence to show the involvement of the serotonin system in autism is the considerable decrease in obsessive compulsive behaviours, anxiety and anger observed in autistic individuals after they receive selective serotonin reuptake inhibitors (104). In the past 10 years many studies have considered the various options of pharmacological treatment for the behavioural aspects of autistic spectrum disorder through considerations of the effects of serotonin and another neurotransmitter, dopamine, on these behaviours (105)(106)(107).

Dopamine

The effects of serotonin levels on behaviours in autistic individuals have been researched in detail; however the effects of dopamine, another neurotransmitter in the brain, have been studied in less depth. Conflicting evidence has been produced from studies assessing the role of the dopamine metabolite homovanillic acid (HVA), in autistic children. Several pieces of evidence showed a difference in levels of HVA in autistic children compared to non autistic children and some evidence showed no difference (73,108,109). The pharmacologic management of certain unwanted behaviours often observed in autistic individuals have shown great results of reducing these behaviours. Dopamine blockers such as haloperidol (110) and pimozide (111) have been shown to reduce hyperactivity, stereotypies and negative behaviours. Neuroleptics which block dopamine and serotonin receptors, such as risperidone (112) have also shown clinical improvement of behaviours associated with autism. Such findings argue for a role of dopamine in autism.

Dopamine is a neurotransmitter that produces pleasure and arousal. Dopamine has many functions in the brain including roles in behaviour, cognition, motivation, reward, inhibition of prolactin production, sleep, mood, attention and learning (113,114). Dopaminergic neurons are primarily present in the ventral tegmental area of the midbrain, the substantia nigra pars compacta and the arcuate nucleus of the hypothalamus (115). Dopamine neurons are essential for tasks such as motor functions, motivation and working memory (116). Another central role for dopamine neurons is the brain reward system, which controls the learning process of many behavioural actions (117,118).

Motor activity, attentional skills, social behaviour and perception of the outside world, which are all implicated in autism and other conditions within the autistic spectrum, are all also modulated by dopamine. The involvement of dopamine in movement control has long been highlighted due to the discovery of the association between dopamine depletion and motor deficits in Parkinson's disease (119). This instigated many clinical investigations into different therapies, such L-Dopa, to improve patient's symptoms. Neuroloeptics were characterised soon after the discovery of dopamine dysfunction in Parkinson's, as powerful dopamine receptor blockers after it was observed that dopamine agonists exacerbate psychosis (120). Dopamine is thought to have a large role in motivation and to the drive of

action. Therefore dopamine is seen to be one of the key transmitters in drug abuse and dependency. An artificial increase in dopamine transmission is the mechanism of action for drugs of abuse that lead to addiction.

Dopamine has been identified as an important neurochemical in prefrontal brain function. PET (Positron Emission Tomography) scans have identified low medial dopaminergic activity in autistic children in comparison to a control group of non autistic children and these findings lead researchers to believe that a dopaminergic deficit may contribute to the cognitive impairment seen in autism, such as the deficit in anticipatory behaviour and difficulty in shifting attention (117)(64)(121).

Reward related dopaminergic effects on learning are well established. Dopamine projections from the midbrain regions, such as the nucleus accumbens, to the frontal cortex and striatum are a part of the behavioural actions that come from reward (122,123). This is supported by the dopamine deficits in Parkinsonism, schizophrenia and drug addiction (124-127). Past evidence has suggested that dopamine neuron activation aids a person in learning to identify the association of particular stimuli with reward (128). Di Chiara and North suggested that the dopaminergic reward system is associated with incentive, preparation of acquiring reward which can be experienced as urgency, thrill and cravings (129). Recent research found evidence for the existence of a relationship between dopamine levels and sensory reward based learning in adults, with results confirming that the effects of reward on sensory processing could be influenced by dopamine (130).

The recent research into the link of dopamine and reward leads to the hypothesis that dopamine is central to the processing of reward, which in turn controls the learning process of many behaviours and actions. The following section develops of the understanding of reward.

1.2i Reward and Neurobiology

The concept of reward is complex. Reward can be perceived as cognitive representations such as novelty, challenge, acclaim, power, money, territory, and security (122). Shultz defines rewards as having three basic functions:

1. Rewards serve as goals for voluntary behaviour. Therefore, rewards can cut short ongoing behaviour and change the priorities of an individual's behavioural actions to more frequently and successfully achieve reward.
2. Rewards have positive reinforcing effects, which mean that the individual gaining reward wants more. Learning seems to progress when rewards occur unpredictably and slows as rewards become more predicted.
3. Rewards produce subjective feelings of pleasure and positive emotional states.

Reward is intimately linked with learning, and through learning, with adjusting behaviours. Reward must also be associated with attention. An individual must be attentive to the environment that a reward may occur in order to achieve the reward. Power and acclaim are forms of complex reward that require cognitive processes to plan for the reward and anticipate it. Processes are then required to recognise if the planning was successful to achieving the reward that was envisaged (131).

FMRI studies in non-addicted individuals have shown that reward motivation and rewards are processed by an interconnecting network of dopamine related brain areas called the 'neural reward system' (132-136). This network comprises of a system made up of the nucleus accumbens, amygdala and the orbitofrontal cortex (137,138). Each of these structures has a specific role in the response to reward. The nucleus accumbens has a number of roles in reward such as responding to the expectation and detection of reward (both the anticipation of reward and the reward itself) and preparing actions in response to the reward. Neurons in the orbitofrontal cortex differentiate amongst different types rewards.

Recent research has also shown that activation in the nucleus accumbens increases with an increased probability of the reward (139). Therefore it is possible to display the activation in the neural systems following an individual's response to a

rewarding stimulus and to approximate the level of perceived reward by measuring the degree of activation of the nucleus accumbens and other limbic structures.

It has been found that there is an increase in dopamine release in the ventral striatum during a reward producing situation (140). The ventral striatum is further linked with reward related information due to its connections with the orbitofrontal cortex and limbic regions like the amygdala (136).
Both the amygdala and the hippocampus project to the nucleus accumbens, which is a component of the ventral striatum. Both the amygdala and the nucleus accumbens demonstrate increased activity during its recognition of pleasure and reward (141).

Dawson et al. carried out a recent study comparing the ability of autistic children, children with Down's syndrome and children with typical development at visual comparisons, novelty preference and visual object recognition (142). Although it was found that autistic children had no problems with visual object recognition, their ability to form rules regarding the relation between a stimulus and a reward revealed a difficulty to produce abstract rules.

As described above, dopamine activation aids learning and is involved in the processing of reward. Therefore, the dopamine deficit seen in people with ASD can affect the learning and reward system, which could explain the problems in processing reward in ASD, demonstrated in the above studies. The characteristics that people with ASD display, such as a lack social interaction and spending time on their own, could be due to these reward processing problems. If the reward from positive social interaction is not felt by an individual, the need to be close to other people will not be felt, which people with ASD, and especially autism, demonstrate. Consequently, the neurobiology of ASD, which includes a dopamine deficit, enables a concept to put forward that the abnormal reward brain circuitry in people with ASD is directly related to the dopamine deficit which is released from those same areas that process reward.

1.2ii Addiction

Individuals addicted to alcohol, drug substances and food, have reward patterns which show an immediate thrill and a desire to obtain the reward from their addiction and these reward patterns have been shown to be dopamine dependent (129). Individuals with ASD display similar reward processing to people with drug addictions.

Natural rewards stimulate the release of dopamine from the nucleus accumbens (143). Similarly, the reinforcing effects of drugs of addiction rely on the generation of dopamine in the nucleus accumbens, which is part of the reward system (144). As described in the section about dopamine, it is a neurotransmitter with multiple properties from motor and cognitive functions, modulating of attention and processing of reward and motivation. The response to drug addiction is not subjected to the adaptive changes that occur in reward related learning, therefore, the drug activates dopamine transmission without decreasing with increasing drug use (143,145,146). This disruption of dopamine activation in the reward areas has been observed in individuals with ASD. It is well recognised that people with ASD have restricted and specific interests, usually only one at a time (147). There are obvious similarities between the single minded tracking down of a single reward and the behaviours of addicts. These similarities and the knowledge that there is a disruption in dopamine function in ASD, links the reward processing of people with ASD along with that of addicts.

1.2iii Functional Magnetic Resonance Imaging (fMRI) and activation of brain areas involved in reward processing

Introduction to functional MRI

Magnetic resonance imaging (MRI) is an imaging technique which is used clinically and for research to visualise the internal structure and in functional MRI displays the functions of the body. MRI allows for a much greater contrast between different soft tissues than computed tomography (CT) which makes it a very useful apparatus for visualising the brain, musculoskeletal system, cardiovascular system and for accurately visualising cancers.

In brain imaging, MRI uses magnetic fields and radio waves to generate two dimensional images of the brain structures without using radioactive tracers, which can then be used to construct 3D images. The MRI scanner contains a magnet which produces a magnetic field approximately a thousand times greater than the earth's magnetic field. The magnet in the scanner is rated using a unit of measure known as 'tesla' or 'gauss' (1 tesla = 10,000 gauss) (148). A typical research scanner has field strength of 3 teslas (149). Hydrogen atoms in the body, which are usually randomly orientated, are forced by the magnetic field to become aligned to the direction of the field. Radio waves are then sent towards the hydrogen atoms, which then bounce off and a computer records the signal. Different tissue types such as fat, muscle, grey matter, white matter and fluid produce different signals and the MRI scanner will produce a 3 dimensional image of the various tissue types. This is due to the varying number of hydrogen atoms in different tissues. The tissue that has the least number of hydrogen atoms, such as bone, is dark in an MRI image, whereas tissue which contains more hydrogen atoms, such as fatty tissue, appears brighter on the image. In order to gain information about the different tissue types, the timing of the radio wave pulses must be changed. Therefore for brain imaging, MRI provides a method of discriminating between grey and white matter, cerebral spinal fluid and other structures in the brain.

Functional magnetic resonance imaging, or fMRI, is a method used to measure brain activity. It works by identifying the changes in blood oxygenation and flow that take place in response to neural activity. When a particular area of the brain is more active it consumes more oxygen which in turn increases the blood flow to the

active area. FMRI can be used to produce activation maps showing which parts of the brain are involved in a particular process (150)(151). Haemoglobin is diamagnetic (repelled by a magnet) whilst oxygenated but paramagnetic (magnetic) whilst deoxygenated (152). This difference in magnetic properties leads to differences in the MR signal of blood depending on the extent of oxygenation. As mentioned above, since blood oxygenation varies depending on the levels of neural activity, these differences can be used to measure brain activity. This form of MRI is known as blood oxygenation level dependant (BOLD) imaging.

It is also important to note the direction of oxygenation change with increased neuronal activity. There is a short decrease in blood oxygenation instantly after neural activity increases, which is known as the initial dip in the haemodynamic response. Following this is a stage where blood flow increases to a level that not only meets the oxygen demand but overcompensates for the increased demand, meaning that blood oxygenation increases following neural activation (153). The blood flow peaks after 6 seconds and then falls back to baseline.

A voxel is a volume of element, representing a value on a regular grid in a three dimensional space. In MRI the unit of analysis is a single voxel, identified by its coordinates x, y and z. By identifying which voxels are significant demonstrates the region of the brain activated by a specific task. The number of voxels activated in a particular region indicates the area activated by a particular task, therefore a high number of voxels in a region of interest indicate highly activated region of the brain.

FMRI as a brain imaging technique has several considerable advantages. The scan is non invasive and does not involve radiation, therefore it is deemed safe for patients. FMRI is also safe and straightforward for the experimenter to use (154). These advantages have made fMRI a popular technique for imaging normal brain function. Over the last decade fMRI has provided new means to investigate a vast number of research areas including how memories are formed, language, learning, pain and emotion (155-157).

FMRI is a useful tool in assessing which areas of the brain are activated after a particular stimulus or activity. For this reason, fMRI has been utilised by many researchers and is being used in this present study.

Some general limitations of using MRI scanning are that high quality images can only be attained if the participant stays very still, however this is not always managed, whether it be due to the patient or due to the task itself, consequently movement errors always have to be taken into consideration. Another few problems that cannot be overcome due to the shape of the scanner are its inability to hold a large participant and that it may cause some people to feel claustrophobic. An MRI is not suitable for people who have been acutely injured, due to the fact that life saving equipment is kept away from the scanner and also because the actual scan takes longer than other imaging techniques which may cause discomfort for participants. A limitation of functional MRI scanning, related to the conclusions that are made about the relation between fMRI and neuronal activity, is that generalisations are often made about individual's brains when the analysis often involves using group averages (158).

Sensitivity and specificity: Sensitivity in terms of fMRI relates to the sensitivity of the group analysis in allowing for unusual individual results (159). A method of ensuring that similarities in a group are apparent and differences are visible is by using the Talairach brain atlas in the analysis, which encourages each individual brain to fit into a model brain, without losing each individual's activation results. Specificity in light of fMRI data analysis is demonstrated by interpreting areas with higher number of voxels in activated areas of interest.

Test repeatability would only be possible if the participants had previously not seen the images in the visual task as memory may affect the brain activation from viewing the images. If they had not seen the images before then the test could be repeated with the same results for each individual participant. However, with a different number of participants, the group results may differ as the average activation demonstrated after the analysis process may differ.

FMRI studies investigating autism and reward

Structural imaging studies have shown that the various anatomical areas in the brain are abnormal in individuals with ASD, such as irregularities in the frontostriatal limbic system, age related changes in grey matter volume and abnormalities in the cerebellar, caudate nucleus, thalamus, amygdala and hippocampal regions of the brain (160-162). Impairments in reward association have also been discovered in children and adults with ASD (163,164). However, only one study has studied the brain's response to reward in people who have ASD using functional magnetic resonance imaging (38). This study used event related functional magnetic resonance imaging to examine the neural activation during reward achievement in individuals with ASD, compared to matched controls. Individuals in the control group showed significant brain activation which included the bilateral anterior cingulate and frontal cortices and also the right insula. Individuals in the autism group showed significant activation in the left anterior cingulate, the middle and superior frontal gyrus and the right parietal lobe. The autistic individuals in this study demonstrated significantly greater activation in the left anterior cingulate gyrus during reward achievement in comparison to the control group. The anterior cingulate gyrus is one of the areas that mediate reward feedback during cognitive tasks, playing an important role in cognitive function such as executive attention, conflict recognition, motivation and arousal (165). In past studies which examined tasks of theory of mind in autistic individuals, anterior cingulate gyrus activation was minimal (40,166). A positive correlation was detected between decreased anterior cingulate gyrus activation and deficits in theory of mind tasks. In the study by Schmitz et al. 2006 the findings of increased neural activation in the anterior cingulate cortex during the attainment of reward in people with ASD may reflect an increased requirement for feedback-related performance monitoring in ASD or an increase in arousal and attention to rewarded stimuli.

An interesting consideration from the results of that study is that since the cognitive area of the rostral anterior cingulate cortex demonstrated increased activation during reward attainment in ASD individuals, it may be due to the increased effort to achieve a desired result by actively directing their goal to an immediate reward. This suggestion can be supported by the evidence that individuals

with autistic spectrum disorder lack the capacity to wait for a reward when they can attain a reward immediately (129,167).

The study by Schmitz et al. 2006 is the only one of its kind which assesses reward processes in people with autistic spectrum disorder in comparison to controls using functional magnetic resonance imaging. However, it only considers monetary reward in the assessment of neural reward functioning in ASD. There are other processes such as visual reward and sensory reward that have not yet been studied in people with ASD using fMRI.

1.3i Reasoning for methodology

International Affective Picture System (IAPS) and visual reward

Researchers have used many methods of investigating emotional states, such as film clips and still pictures (168). A widely employed set images used for visually testing emotions is the International Affective Picture System (IAPS) (169). Complex images in the IAPS database demonstrate emotion evoking images of different weightings. The goal was to demonstrate a large set of standardised, emotionally evocative colour images that can be easily accessed and used in research. The standardised set of images have been rated between 1-10 in their ability to provoke valance (unpleasant/pleasant, 1-10), arousal (calm/excited, 1-10) and dominance (dominated/in control, 1-10). Neutral images have scores approximately around 5. The study by Lang consisted of 100 adult volunteers, which was a limitation to the Lang study, however other studies have carried out the same experiment to verify the results on more volunteers (170,171). The original IAPS study by Lang divided the results into adult female, adult male and then adult male and female together. There was then a separate table with results from the group of children. This validation process allows researchers to use all groups of images in their studies, or just particular groups of images, such as those validated by male and female adults, which is the validated group of images used for this study. The reason for using this group instead of just the male validated images was due to the knowledge that the future expanded study may include female participants; therefore the joint male and female adult validated images were employed.

Work has been done to identify the difference in the emotional processing and difference in brain activation in fMRI between images of emotional faces and IAPS images (172). Although this study assessed the sets of brain regions that were activated in processing facial and the IAPS images, this study utilised IAPS images depicting emotions such as sadness, anger, happiness, which were selected by the researchers. However, work has not been carried out to identify which brain areas are engaged from complex combinations of different emotionally weighted IAPS images. The images selected for this study from the IAPS database demonstrated pleasure and displeasure. However the IAPS database does not allow for the images to be split simply into two groups, therefore three pleasure variants, which

demonstrated pleasurable images in different forms, and one displeasure group of images was selected. The groups of images were divided into the following:

- Control images (neutral)
- Aroused, in control, pleasure (high valance, high arousal, high dominance)
- Non aroused, in control, pleasure (high valance, high dominance, low arousal)
- Aroused, not in control, pleasure (high valance, high arousal, low dominance)
- Displeasure (high arousal, low valance, low dominance)

The images were not selected by simply viewing the images, instead the rating of reach of the emotional categories (valance, arousal, and dominance) was set for each of the five groups (stated above) and the images were selected via the ratings, which prevented researcher bias in the choice of visual stimuli.

In addition research has not been undertaken to study the effects of these emotional stimuli on the reward circuitry in the brain. These complex combinations of rated emotional images can reveal the impact of various emotionally rewarding and non-rewarding stimuli on the reward circuitary in the brain. With areas of the reward circuitry such as the amygdala and the nucleus accumbens seen to have deficits in people who have ASD, testing a hypothesis that emotion evoking images from IAPS database activate areas known to process reward in normal participants, could allow for a hypothesis that people with ASD process reward in a different manner to the non ASD population. This hypothesis could be extended to investigate individuals with ASD and observe whether the areas of their brains activated from these emotionally charged images and other rewarding stimuli, differ to the areas of activation in controls due to known deficits in brain reward areas in ASD.

1.3ii Aims for the study and long term objectives

Specific aims for this study:

- Developing the four paradigms; gambling, visual, touch and materials, and exploring how best to apply the paradigms in a functional MRI setting.

- Focused development of the visual paradigm using the IAPS image database, with the aim of creating a task which assesses positive emotional stimuli (rewarding images) and negative emotional stimuli. The advantages of focusing on this visual paradigm are that through investigating which groups of images activate which area in the brain, we can not only explore the differences between the groups of IAPS images, but we can also identify which areas of the brain are activated during rewarding stimuli. This information is beneficial when developing the project and comparing the brain activation in an ASD population and a normal population.

- To assess which anatomical and functional area of the brain is significantly activated ($p < 0.05$) from each of the groups of images as stated above in the control participants and the ASD participants.

- To assess the similarities and differences between the neural areas activated from the different image groups in the control participants and the ASD participants.

- To assess whether areas in the reward circuitry of the brain, including the amygdala and the nucleus accumbens are activated from visualising rewarding images in the MRI scanner.

- To explore for the similarities and differences in which area of the brain is activated for a particular image category, between the control group and the ASD group.

- Investigate the differences between the ASD participant and a control participant in brain region activation, allowing for the exploration of the hypothesis of abnormal reward circuitry in ASD, aiding the development of a larger scale project.

Long term aims

The long term aims of the project are to examine the neural activation during the visualisation of images from the IAPS library, differing in emotional weightings, along with the testing of sensory reward through two touch paradigms and risk taking and risk aversion reward by using a gambling paradigm in ASD participants

compared to controls. The reasoning for these different reward testing paradigms is stated below:

- ***Hyper/hyposensitivity to pleasurable sensory stimuli***

It is recognised that a large proportion of individuals with ASD suffer from hyper/hyposensitivity (88). This includes sensitivity to temperature, noise and touch (173,174). As mentioned in section 1.1ii, individuals with ASD have been found to have altered c-fibre functioning (88,89). This information, along with findings of a class of c fibres in hairy skin that respond to pleasant stroking touch (91), allow the development of the theory that the hyper/hyposensitivity in autistic individuals could be due to deficits in c-fibre responses. This leads to a hypothesis that individuals with ASD may have deficits in the experience of sensory stimulation. In order to test this hypothesis, a stroking stimulator producing a soft and light stroking action can be applied to the hairy skin of individuals with ASD whilst they have a functional MRI scan.

Along with the hyper/hyposensitivity to noise, temperature and soft touch, people with ASD often have a great like or dislike to an everyday fabric type (175,176). For this reason, a hypothesis can be built regarding whether everyday materials cause an abnormal brain activation pattern during fMRI in the brains of people with ASD in comparison to controls.

- ***Gambling reward***

A study was carried out examining the neural circuitry associated with immediate versus delayed reward processing in adults with attention deficit hyperactivity disorder (ADHD) (177). The results of the study demonstrated that in people with ADHD, a delay of rewards produces hyper activity in the neural circuits involved in motivation, emotion and reward, which could contribute to the intolerance of delays in reward associated with ADHD. It is known that individuals with ASD also demonstrate impairments in the brain regions involved in motivation and emotion, along with people who have ADHD (167). Therefore, it is acknowledged that individuals with ASD have difficulties with the ability to wait for a reward that they can attain. This piece of information is vital in understanding how people with autism process rewards.

The gambling task described in Rogers at al. 2003 (178) is a decision making task in which participants decide between two risky gambles in order to maximise monetary reward and then have a short period of time to process the outcomes of their choice, whether they be positive or negative.

A hypothesis can be formed that since individuals with ASD have difficulties in waiting for reward, in a gambling situation they must choose an option that allows them to win their reward straight away, regardless of what it is and whether their reward can increase if they wait. The gambling task above allows this hypothesis to be investigated in an fMRI scanner in order to visualise the brain areas of activation during the decision making phase and following the attainment or loss of a reward.

Ethical approval:

Ethical approval for this study was approved by the Stockport research ethics committee with the following documents reviewed and approved by the committee:

Document	*Version*	*Date*
Application	5.4	17 September 2007
Investigator CV		
Protocol	1	17 September 2007
Summary/Synopsis		17 September 2007
Compensation Arrangements	1	31 December 2006
Interview Schedules/Topic Guides	Personal Hobbies & Interests	
Questionnaire: The Adult Autism Spectrum Quotient (AQ) ages 16+		
Questionnaire: Demographic and background information		
Questionnaire: National Adult Reading Test (NART)		
Questionnaire: BDI-II		
Participant Information Sheet: Control group	3.02	16 November 2007
Participant Information Sheet: ASD volunteers	3.02	16 November 2007
Participant Consent Form	2.2	16 November 2007
Response to Request for Further Information		

The REC reference number is 07/H1012/72.

Recruitment:

- The aim for the recruitment of controls was to enlist 15 male participants who have no known autistic spectrum disorder or psychiatric medical condition and who are not taking any antidepressant or antiepileptic medication. An advertisement was placed in the University of Liverpool intranet which was made up of a brief explanation of the study and contained contact details for the researchers running the study which enabled anybody interested to ask and questions and gain more information.
- The aim for the recruitment of non control participants was to have 15 participants who have high functioning autism or Asperger's syndrome, who have a normal or above average IQ and who are also not taking any antidepressant or antiepileptic medication. The reasons for recruiting participants with above average IQ was in order to ensure that all the participants would understand the tasks that they would undertake during the

scan. An advertisement was placed in the Chester ASD carers support group newsletter, the Northwich ASD carers support group newsletter, the Halton ASD support group newsletter, and the CASPAR (Cheshire Asperger's Syndrome Parents' Action for Resources) newsletter. All of the above support groups allowed the opportunity for the researchers and I to take part in their group sessions, where we used that time to inform carer's of adults with ASD about the study and to answer any questions that they had.

Participants:

1 male individual aged 22 with ASD (diagnosed by ICD10 criteria) was recruited. 7 male controls between the ages of 18 - 40 years of age were also recruited from Liverpool University by advertisement. On responding to the advertisement each individual was provided with a detailed information sheet. A member of the research team was available to answer any questions that occurred. On receiving a verbal consent form from the candidate, a preliminary meeting was arranged for signing of the written consent form and Phase 1 of the study.

Evolution of methodology:

Throughout the development of this study, changes have been made to the methodology to enhance the validity of the methodology:

- The initial visual paradigm consisted of 45 images of three kinds: those reported as rewarding by ASD subjects (5 images); those deemed rewarding by a non-ASD population from the International Affective Picture System IAPS library (20 images); and those deemed to be neutral by a non-ASD population also from the IAPS library (20 images). It was required for the paradigm to be repeated, however, if the same image set was to be shown to the participants, the problem would arise of the participants remembering the images and the rewarding factor of the image may be overridden by memory of the image. Therefore it was necessary to repeat similar rated images from the IAPS database. The final visual paradigm consisted of those reported as rewarding by a non-ASD population (60 images), those reported as neutral by a non-ASD population (60 images) and those deemed rewarding by ASD subjects (15 images which replace 15 of the neutral images for the ASD participants). The images deemed as rewarding and non-rewarding by a non-

ASD population were selected from the IAPS database and the images were selected from their ratings as described in methodology below. The full list of the images used for the final visual paradigm is located in appendix 1. As the number of images for the final paradigm was increased to 120 in total it was decided that the questionnaire to rate each individual image should not be included in the paradigm.

- The touch paradigm/processing of sensory reward began with a lateral stroking stimulator which would be stroked lightly on the forearm and the palm skin of the participant. Each stimulus probe was to stroke a 3-5 cm long chord of skin at about 3 cm/s. The probe was to consist of two types of surfaces, a soft surface (such as a cosmetic brush) and a rough surface (such as fine plastic mesh). In order to test soft touch in detail it was decided to exclude the rough surface probe from the paradigm. Instead, the soft probe was to be tested in active and passive touch, on the skin of the forearm and the skin of the palm of the participant. The area of skin to be brushed with the probe remained at 5cm long chord of skin and the rate remained at 3cm/s. The timings for the paradigm required for the skin to be brushed for a long enough time period for activation to be apparent on the fMRI scan data, therefore the 'on' period for brushing to be undertaken was for 9 seconds. The 'off' period of a rest was for 6 seconds to allow any activation to end. The different areas of skin and the active/passive touch were repeated 8 times in varying combinations. This paradigm was based on similar work done by Francis Mcglone and Rochelle Ackerley (179). An additional part to the sensory reward paradigm was the materials section, which consisted of four fabrics placed individually in the palm of each participant to allow them to feel the fabric with their fingers and thumb. The fabrics were cotton, wool, satin and silk. This paradigm was added due to the increasing knowledge that individuals with ASD have sensitive sensory systems and often have a great dislike or an immense like of particular fabrics (180,181). The timings for this paradigm had to be adapted so that enough time was given for adequate activation to be shown on the scans but also to make sure the overall scanning time was less than 1 hour long as recommended by the research institute where the scanning took place.

- In the original methodology, after the participants carried out the tasks in each paradigm in the MRI scanner, they were to experience the same paradigms in the Magneto-encephalography (MEG) scanner. Due to the knowledge that the fMRI data alone would demonstrate the areas in the brain that are activated during the testing of the paradigms, along with problems with the MEG scanner in the research institute, it was decided that the participants should not undergo a MEG scan.

PHASE 1: Screening questionnaires.

To ensure validity of the diagnosis of ASD, to profile the level of autistic trait in controls and cases and to allow for confounding variables, the following questionnaires were completed by each participant in the study:

1. Standard demographic information e.g. educational and employment history (these were used as proxy measures of adaptive functioning). This included data on the candidate's suitability for MRI scanning using a standardised questionnaire. Data was also collected regarding the use of medication /drugs both prescribed and not prescribed.
2. The National Adult Reading Test (NART). This gives an approximate IQ. This has good reliability and is largely resistant to the effects of neurological and psychiatric disorders (182).
3. Previous/current psychiatric history. This is to allow screening for significant confounding psychopathology (e.g. schizophrenia).
4. A brief 'hobbies and interests' screening interview/questionnaire'. This has been developed from the ADI-R (The autism diagnostic interview) (183-185). A well validated tool used for the diagnosis of ASD. This questionnaire was used to ascertain circumscribed interests in the ASD group. This information was used to design the research paradigm (see below).
5. The Autism Quotient for Adults (186). This questionnaire has been developed to measure the degree to which an adult with normal intelligence has autistic traits. The results indicate that it has good discriminative validity and good screening properties at a threshold score of 26. It also gives a spectrum score with normative data which allows an individual to be placed against the normal population for his or her degree of autistic trait.
6. The Becks Depression Inventory (187-190). It has been shown that clinical depression affects cerebral functioning in areas related to reward processing (191). Depression therefore represents a confounding variable for the present study.

At the end of Phase 1, exclusion and inclusion criteria were applied.

During recruitment, subjects were informed of the exclusionary criteria.

I. ASD volunteers.

Inclusion:

- Proven diagnosis of ASD with an AQA score consistent with this diagnosis.
- Absence of co-morbid psychiatric disorders.
- The presence of unique reward triggers
- IQ $\geq$100
- Informed consent
- Age 18-40 years
- Male gender

Exclusion:

- Presence of co-morbid psychiatric conditions
- Absence of unique reward triggers
- Presence of any implants which would contraindicate the use of MRI technologies (see above).
- The use of any psychotropic medication either prescribed or not prescribed.
- IQ <100
- Individuals who do not have a circumscribed interest that can be presented visually.

II. Non-ASD volunteers

Inclusion:

- The absence of any evidence of ASD through use of the AQA questionnaire
- The absence of any psychiatric disorder through use of the Beck's depression inventory questionnaire
- The absence of any implants which would contraindicate the use of MRI technologies (see above).
- IQ $\geq$100
- Informed consent
- Age 18-40 years
- Male gender

Exclusion:

- The presence of ASD
- The presence of a psychiatric condition
- The use of any psychotropic medication either prescribed or not prescribed
- IQ <100

Following recruitment, the information gathered during PHASE 1 from the volunteers was used to prepare the visual stimuli used in PHASE 2 – processing visual reward (see below). The circumscribed interest's images from the ASD group consisted of fifteen rewarding images that were chosen from the answers given in the hobbies and interests questionnaire. These 15 images were displayed during the visual paradigm in exchange for fifteen of the neutral images displayed for the control group.

PHASE 2: Functional imaging of reward processing:
4 paradigms were presented to each individual whilst they were in the fMRI scanner. The paradigms using Presentation software (Neurobehavioural Systems, Inc, Albany, CA http://www.neurobs.com/) were projected to them onto a mirror (located on the HR head coil which was positioned around their head in the scanner) via a projector at the back of the room.

fMRI acquisition

fMRI: The scans were carried out in the Magnetic Resonance and Image Analysis Research Centre (MARIARC) at the University of Liverpool. Participants underwent a 7 minute structural scan (MPRAGE: 176 slices, TR = 2040ms, TE = 5.57ms). Functional images were collected sing an EPI sequence (TR = 3000ms, TE = 30ms, Resolution = 3x3x2.5mm, slice thickness = 2.5mm, gap = 0.5mm, number of slices = 42). Participants were given instructions about the tasks and they had an opportunity to practice the gambling task before the task. The participants lay on a padded scanner bed, with earplugs and foam cushions next to their head to avoid excessive movement. The computer display was projected onto a screen at the back of the MRI scanner and the participant viewed the display through an angled mirror on the head coil positioned over their head. Responses to the gambling paradigm were made on a button box placed under the participant's hand.

Below is an image of a similar 3 tesla scanner by Seimens, with the head coil in place for brain imaging. For our participants, a mirror is placed above the head coil in order for them to be able to see the images projected onto a screen behind them.

(192)

The participants were laid flat on the scanner bed, with their heads resting in the head coil. Underneath their arms were sand bags which aided them in positioning their arms comfortably for the tasks. After placing earplugs in their ears, the participant's heads were stabilised using soft cushions on either side of their head in the head coil.

Standardisation

Standardisation of all aspects of the study was demonstrated in various aspects of the study.

- All of the participants were asked to fill in the questionnaires at a meeting which took place 1 month or less before the scan date.
- The meeting before the scan and the actual scan itself was undertaken by the same person in order to ensure that there were no differences in how each parts of the study were carried out. The same radiographer was also present for every scan to ensure that the MRI values were the same for each participant.

- The instructions given to the participants regarding the tasks for them to undertake during the scan were read from a standard instruction sheet, which ensured that every participant was given exactly the same instructions.

Paradigm 1: Gambling Paradigm (20 minutes)

The gambling paradigm was a decision making task (193-195). It consisted of a number of trials, which involved the participants choosing between playing one of two simultaneously presented gambles. Each gamble was represented visually by a histogram, the height of which indicated the probability of winning a number of experimenter-defined points. The possible gains were indicated in green text above the histogram, and the possible losses were indicated in red text underneath. One of the gambles (yellow) was a control gamble, consisting of a 0.5 probability of winning 10 points and a 0.5 probability of losing 10 points. The alternative 'experimental' gamble (blue) varied in the probability of winning which was either high or low (75 vs. 25%), the expected gains which were either large or small (80 vs. 20 points), and the expected losses which were either large or small (80 vs. 20 points).The combination of these variables, in a completely crossed design, resulted in eight trial types (see Table 1).

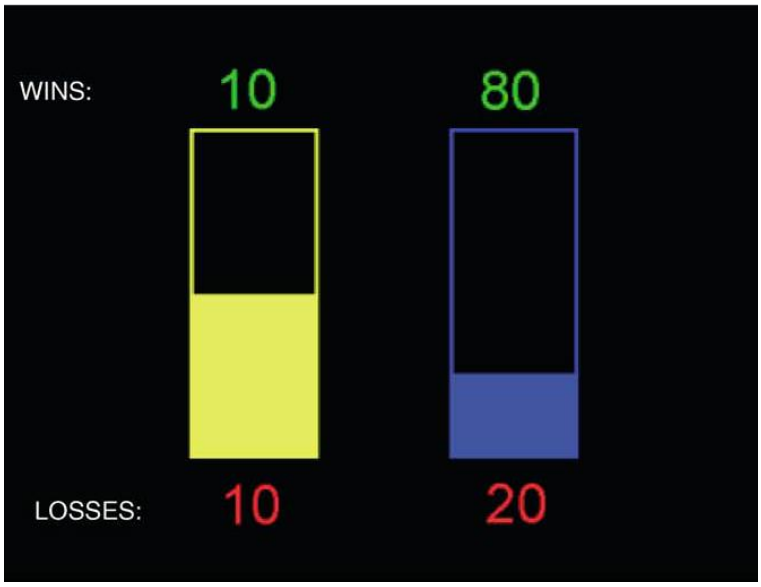
Table 1 - Eight Types of 'Experimental' Gamble Resulting from the Combination, in a Crossed Design, of Two Levels of Probability, Expected Gains, and Expected Losses.

Probability	**Expected gains**	**Expected losses**
High 0.75	Large (80)	Large (80)
		Small (20
	Small (20)	Large (80)
		Small (20)
Low 0.25	Large (80)	Large (80)
		Small (20)
	Small (20)	Large (80)
		Small (20)

The control gamble and the 'experimental' gamble appeared randomly on the left or right of the display. The volunteer was required to press the '1' or the '2' key on a standard computer keyboard to indicate choice of the gamble presented on the left or the right. The dependent measure was the proportion of choices of the 'experimental'

over the control gamble as a function of the combination of probability, the size of expected gains, and the size of the expected losses in the 'experimental' gamble. Figure 1 shows a trial from the decision-making task consisting of an 'experimental' gamble with a 25% chance of winning 80 points and a 75% chance of losing 20 points vs. the control gamble with a 50% chance of winning 10 points and a 50% of losing 10 points.

Figure 1 - Example display of a trial of the decision making task (193).



Paradigm 2: Visual Paradigm (10 minutes)

This consisted of 120 images displayed on a computer screen visible to the subject picked from the International Affective Picture System (www.phhp.ufl.edu/csea/Media.html). The images were scored by 3 categories, each value 1-10; valance (not pleasurable to very pleasurable), arousal (calm to feeling very excited) and dominance (feeling dominated to feeling in control). The images in the paradigm were of three kinds: those deemed to be neutral by a non-ASD population also from the IAPS library (60 images), those deemed as having a positive (rewarding) emotional value or negative emotional value by a non-ASD population from the IAPS library (60 images) and images of each ASD participant's circumscribed interests (Replaces 15 of the neutral images for the ASD participants). The images which were deemed as neutral by a normal population had equal scores for all 3 categories (between 3.5 - 5.5 on average). Images which were classed as rewarding fell into one of the following categories:

The groups of images were divided into the following for our study:

- Neutral - .e.g.: policeman, cowboy, snake bees.
- High Valance (pleasant) & High Arousal (exciting) & High Dominance (in control) – e.g.: attractive female, erotic couple, skier, sailing. This combination of high valance, high arousal and high dominance was chosen as this combines high ratings of all of the categories that were rated on emotion content in the IAPS images.
- High Valance (pleasant) & High Dominance (in control) – e.g.: seal, kittens, baby, and butterfly. This combination of categories was chosen as they demonstrate visual stimuli that are pleasurable but also make the person viewing them feel in control and this combination of emotions has not been investigated in an fMRI study before.
- High Valance (pleasant) & High Arousal (exciting) – e.g.: waterfall, cliff divers, parachute, and rollercoaster. These images are different to the high valance, arousal and dominance as these images have a low dominance score which means that the person viewing the images will find the images pleasurable and exciting but feel like they have little control over the theme of the images.
- High Arousal (exciting) – e.g.: gang, starving child, black eye, war. These images have a high arousal rating which means that they were deemed as exciting or shocking to the viewer. However they also contain a low valance score which means that the images have a low pleasure rating, and also a low dominance score which means that the viewer feels a lack of control.

The rationale for the various groupings of images was to demonstrate groups of images that contained positive (rewarding) emotional stimuli of varying degrees, along with negative emotional stimuli, which consisted of the high arousal images. This would aid us in gathering information on which rewarding images activated which brain regions and the differences with the activation created from the negative emotional stimuli. The IAPS database has been validated by a normal study population and the images were rated by each individual, with the mean ratings of each image displayed in a table. The images were validated by a large number of individuals, however some of the neutral images, for example, could have been rated

as rewarding by particular individuals, but the average score of the image places the it into the category of neutral in our study. As our study contained few participants in comparison to the number of individuals used to validate the IAPS images, some of the neutral images may have been identified as rewarding to some participants. This information must was considered whilst analysing the data and comparing brain activation from a high emotional value group of images and the neutral images. Images of each type were randomly distributed through the display. For the ASD participants 15 of the neutral scored images were exchanged with images of the participant's circumscribed interest images. The same neutral images were replaced for the circumscribed interest images for each of the ASD participants. A list of the images and their individual IAPS score is located in Appendix A. The sequence of the exposure of each of the images, the fixation cross and their timings is listed in Appendix B Functional brain imaging was performed during this viewing.

Paradigm 3: Touch Paradigm (8 minutes)

This paradigm relates to the processing of tactile sensory reward. Stroking of the skin by textured surfaces will be carried out as described in a previous study by Olausson, Cole, Vallbo and McGlone 2008 and further studies researching brain activation from various forms of touch (196,197)(88,198). Two identical lateral stroking stimulators were used to stroke the skin lightly proximo-distally on the volar forearm and palm. This device was a soft cosmetic brush. The participant held the brush in their right hand and the researcher held the second brush in their hand. The MRI presentation screen gave directions for the participant to stroke their left palm or their left arm and then gave directions for the researcher to stroke the participant's left palm or left arm. These four tasks were repeated eight times in various orders. Each brush was stroked over a 3-5 cm long chord of skin at about 3 cm/s. The tasks lasted for 9 seconds each in which the participant continued to stroke the skin for 9 seconds. A rest period of 6 seconds followed each task. Functional brain imaging was performed during this task.

Paradigm 4: Material Paradigm (8 minutes)

To complete the touch paradigm the participants were asked to feel four materials that were placed into their left hand by the researcher: satin, silk, cotton and wool. The researcher placed the material on the participant's fingers so that they

could rub the material in between their fingers and thumb. The task was carried out for 9 seconds, following which there was a break of 6 seconds. The timing was managed by the researcher who had a timed presentation illustrating which material to place in the participant's hand, in which order and for how long. Each material was placed in the participant's hand 8 times in various orders. The participant was unaware of which material was being placed in their hand. Functional brain imaging was performed during this task.

After this task the participant should rated pleasantness to touch on a scale from 0 to 10.

fMRI Analysis

fMRI data analysis was carried out using Brain Voyager QX software version 1.3 (199). Due to time constraints the present stud investigates the visual paradigm fMRI results only. The following data pre-processing steps were utilised for the visual paradigm: (1) 3D motion correction, (2) slice scan time correction, (3) spatial smoothing, (4) temporal filtering. The control group visual paradigm fMRI data was split into 5 conditions: (1) neutral, (2) valance & arousal, (3) valance, arousal & dominance, (4) arousal, (5) valance & dominance. Anatomical and functional volumes were co-registered and normalised to Talairach space.

The Talairach coordinate system of the human brain, created by neurosurgeon Jean Talairach, uses landmarks to adjust the orientation, position and size of an individual brain to match a reference brain. This then allows for the description of the location of brain structures, independent from individual differences in size and overall shape. (200)(201)

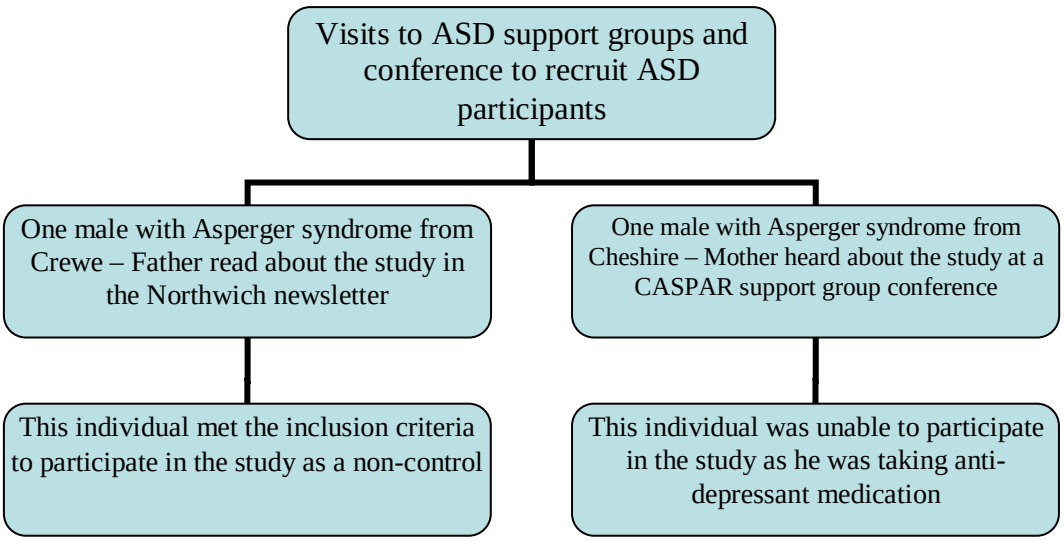
A general linear model multi factor approach was then applied, placing all of the control participant's data together in order to create a batch analysis for the control group. The same steps were then carried out for the ASD participant and a general linear model single factor approach was applied to the data. A p-value < 0.000012 and a cluster threshold of 50 voxels were used in order to find significant activation in the control group batch analysis. A p-value < 0.001603 and cluster threshold of 50 voxels were used in order to find significant activation in the ASD participant and control participant comparison.

Recruitment

ASD participants: Recruitment of participants with ASD involved me visiting carer support groups around the Merseyside and Cheshire regions in order to inform people about the study, to answer any questions that carers at the support group may have had about the study and to try and recruit participants who fit the inclusion criteria. The following is a list of the support group sessions that I attended along with my supervisor in order to speak to carers about the study:

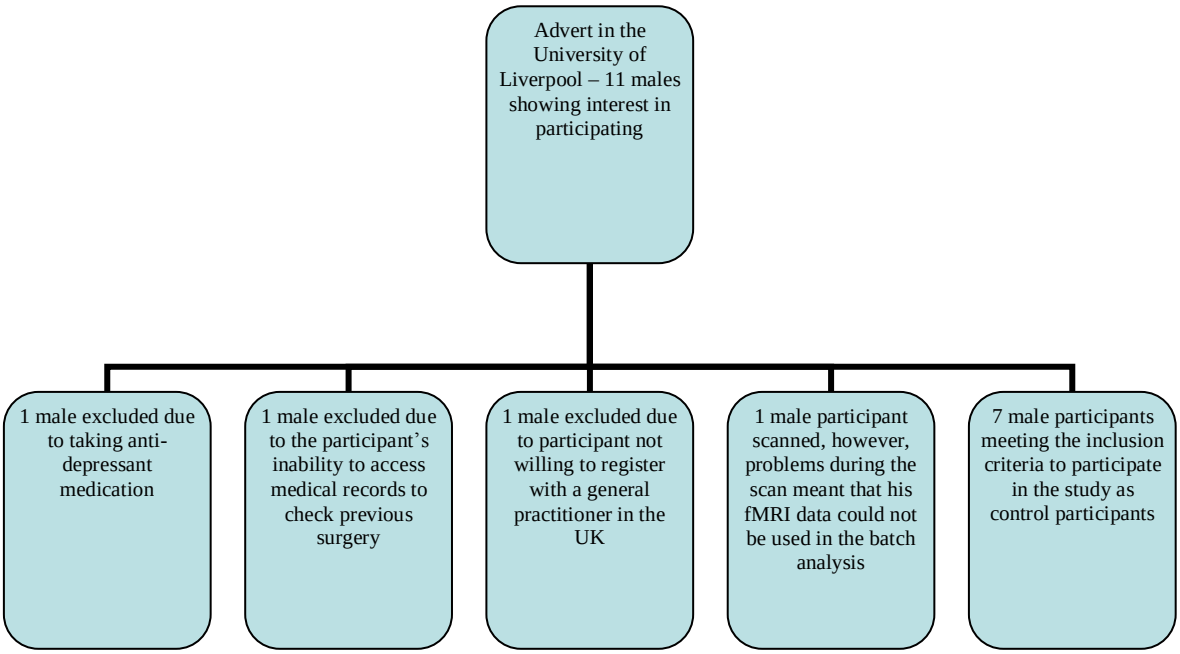
- Chester County ASD Support Group 7pm, 25th February 2009.
- Northwich ASD Carers Support Group 12pm, 1st April 2009.
- Halton Autistic Families Support Group 7pm, 16th April 2009.
- CASPAR (Cheshire Asperger's Syndrome Parent's Action for Resources) autism conference for parents, carers and professionals 9-4, 21st May 2009.

Following these meetings contact was maintained with the organisers of the groups in order to reinforce the information about the study and to update them on any changes in the inclusion criteria for participants. An advert was also placed in the University of Liverpool intranet site for participants with ASD. This advert was renewed every month. Attending the above meetings led to the following people showing interest in participating as an ASD participant in the study:



Therefore, this information demonstrates that there was one individual interested in participating in the study that fit the inclusion criteria.

Control participants: The recruitment of control participants involved placing an advert in University of Liverpool intranet announcements page, with information about the study and contact details for anyone interested to ask for more information. The following diagram demonstrates the number of people who showed interest in participating as a control in the study:



The problems with recruitment and the limitations to this study are discussed in the limitations section.

Phase 1 results

Participant recruitment - The initial control group number was 11 participants, however various circumstances arose which lowered the control group to 7 participants who were included in the final analysis. The circumstances that arose are listed below:

- A participant was unable to participate as he was taking anti-depressant medication at the time of the study, which is one of the exclusion criteria for our control participants.

- A participant was unable to access his old medical records, which was required to give information about a past ear surgery. As the information could not be accessed, he was unable to participate due to health and safety reasons in the scanning centre.
- A participant was interested in participating in the study, however as he was an international student studying in the city for a year he was not registered at a general practitioner and was not willing to register, which was necessary for the health safety regulations of the scanning centre.
- A participant was scanned and all of the tasks were carried out, but due to complications in the scanning time (as he was the first participant to be scanned) his data could not be included in the final analysis.

The recruitment of ASD participants for the case group was more difficult than initially expected. Although many support groups were visited and many adverts were put into newsletters and on the University intranet, there was a lack in people showing interest in participating. The initial group number could have been 2 participants, however one of the individuals with ASD was taking anti depressant medication at the time of the study which is one of the exclusion criteria for the ASD group as it is for the control group.

Participant characteristics Control participants were 7 males between the ages of 19 and 24 (mean age 22 years). None of the participants were taking any anti-depressant medication at the time of the study and had not been taking any anti-depressant medication for a year previous to that. All of the control participants had no known diagnosis of Asperger's syndrome or high functioning autism.

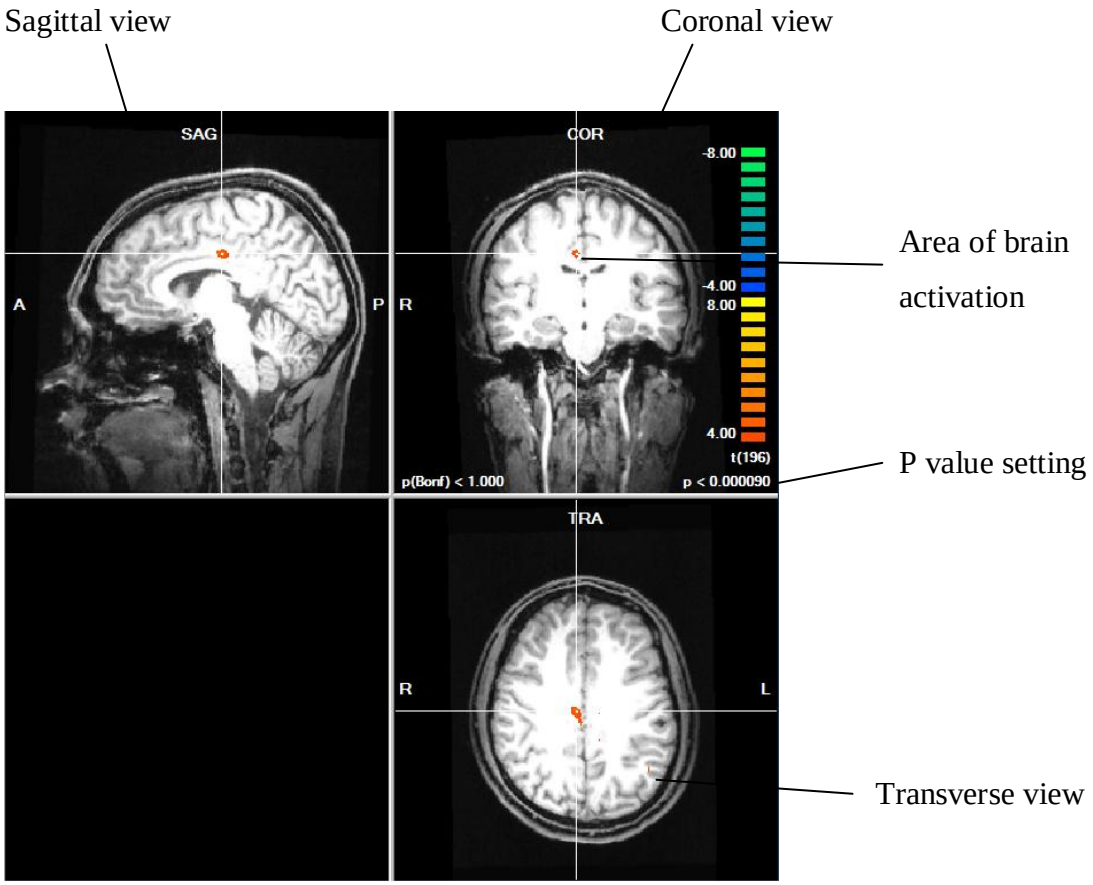
The ASD participant recruited was aged 22 years, had never taken anti-depressant medication and was diagnosed with Asperger's syndrome four years prior to participating in this study.

Phase 2 results – Visual paradigm fMRI results for control group batch analysis

The control participants were grouped together in a batch analysis which averages across the group. This allowed for the main areas of activation amongst the individual control participants to be demonstrated. The areas of activation at a p-value < 0.000012, in each condition of the visual paradigm, were

localised and are listed below. In the five conditions of the visual paradigm, similarities in areas of activation for all of the conditions were evident from the control group results. The p-value was set at < 0.000012 for all of the conditions which was the lowest p-value that allowed activation to be observed in all five of the conditions. Common areas of activation were in the frontal lobe, particularly the medial frontal gyrus, the limbic lobe and the anterior cingulate gyrus.

Below is a diagram of a post-analysis fMRI image. There are 3 views which can each be regulated in order to find areas of activation in the brain. The p-value setting at the bottom right of the coronal view is adjusted by increasing or decreasing the threshold, which translates to decreasing p-value (increasing threshold) and increasing p-value (decreasing threshold). Once the p-value is set, the areas of activation can be located as they will 'light up', which means that areas of the brain will show coloured spots as demonstrated below. As the p-value increases however, the area of the brain 'lit up' or activated increases, therefore the area activated at a lower p-value will be small but will have the greatest significance. The 3D volume tools allow for an activated region of interest to be pin pointed in order to reveal the number of voxels in the activated region.



Neutral images. Significant areas of brain activation from viewing the neutral images are listed in table 2.

Table 2 Neutral images: Area, voxel size related to the largest cluster of activation in each anatomical region at p value < 0.000012, Talaraich coordinates of clusters of activation in the group analysis of control participants.

Anatomical brain region	Voxel size	Coordinate x	Coordinate y	Coordinate z
Right frontal lobe, medial frontal gyrus	511	-17	31	27
Left limbic lobe, anterior cingulate gyrus	181	-15	7	30
Left frontal lobe - cingulate gyrus	142 209	-19 -16	24 14	20 37
Left temporal lobe, middle temporal gyrus	164	-37	-75	21
Left occipital lobe, lingual gyrus	140	-6	-70	5

Valance, Arousal & Dominance images. Significant areas of brain activation from viewing the high valance, high arousal and high dominance images are listed in table 3.

Table 3 Valance, Arousal & Dominance images: Area of activation, voxel size related to the largest cluster of activation in each anatomical region at p value < 0.000012, Talaraich coordinates of clusters of activation in the group analysis of control participants.

Anatomical brain region	Voxel size	Coordinate x	Coordinate y	Coordinate z
Right frontal lobe - superior frontal gyrus	100 132	24 11	-42 27	36 44
Right limbic lobe, anterior cingulate gyrus	122	19	27	25
Left parietal lobe, precuneus	170	-12	-60	33
Left frontal lobe, medial frontal gyrus	199	-14	29	30
Left temporal lobe, supramarginal gyrus	170	-50	-48	23

Valance & Dominance images. Significant areas of brain activation from viewing the high valance and high dominance images are listed in table 4.

Table 4 Valance & Dominance images: Area of activation, voxel size related to the largest cluster of activation in each anatomical region at p value < 0.000012, Talaraich coordinates of clusters of activation in the group analysis of control participants.

Anatomical brain region	Voxel size	Coordinate x	Coordinate y	Coordinate z
Right frontal lobe	396	44	19	21
Left frontal lobe	79	-27	23	25
Left limbic lobe, anterior cingulate gyrus	42	-18	29	23
Left occipital lobe, fusiform gyrus	15	-44	-66	-11

Valance & Arousal images. Significant areas of brain activation from viewing the high valance and high arousal images are listed in table 5.

Table 5 Valance & Arousal images: Area of activation, voxel size related to the largest cluster of activation in each anatomical region at p value < 0.000012, Talaraich coordinates of clusters of activation in the group analysis of control participants.

Anatomical brain region	Voxel size	Coordinate x	Coordinate y	Coordinate z
Right frontal lobe	238	23	-41	34
Right parietal lobe, precuneus	242	19	-47	49
Right limbic lobe, anterior cingulate gyrus	185	12	-2	41
Left frontal lobe - medial frontal gyrus - precentral gyrus	 167 342	 -24 -47	 -1 -5	 43 11
Left parietal lobe, precuneus	222	-37	-32	37
Left insula	348	-38	-24	21
Left temporal lobe, precentral gyrus	187	-56	-11	12

Arousal images. Viewing of the high arousal images resulted in significant clusters of activation in the areas listed in table 6.

Table 6 Arousal images: Area of activation, voxel size related to the largest cluster of activation in each anatomical region at p value < 0.000012, Talaraich coordinates of clusters of activation in the group analysis of control participants.

Anatomical brain region	**Voxel size**	**Coordinate x**	**Coordinate y**	**Coordinate z**
Left frontal lobe - medial frontal gyrus	12 16	-18 -14	28 26	17 30
Left limbic lobe, anterior cingulate gyrus	5	-18	32	23

The areas of activation were compared between the conditions in and the differences in activation between the conditions are demonstrated in table 7.

Table 7 Brain regions activated for each specific visual condition and the voxel size for the largest cluster of activation in the area at p value < 0.000012.

Brain Regions	**Visual Paradigm Conditions**				
	Neutral	***Valance, Arousal & Dominance***	***Valance & Dominance***	***Valance & Arousal***	***Arousal***
Left frontal lobe	209	199	79	342	16
Right frontal lobe	511	132	396	238	
Left temporal lobe	164	170		187	
Right temporal lobe					
Left limbic lobe	181		42		5
Right limbic lobe		122		185	
Left parietal lobe		170		222	
Right parietal lobe				242	
Left occipital lobe	140		15		
Right occipital lobe					
Left fusiform gyrus			15		
Right fusiform gyrus					
Left insula				348	
Right insula					

The predicted areas of activation for each of the different groups of images compared to the actual activation for each image group is demonstrated in Appendix C.

Case vs. Control comparison

An age and gender matched control was selected from the control participant group, to allow a comparison to be made regarding the fMRI data results of the ASD participant and a control participant. Just one control participants was chosen to carry out the case vs control comparison instead of using the whole participant group data, as the differences between individual participants' means that it would not be a fair comparison to compare the case to an averaged set of data from the group of controls. The tables below demonstrate the anatomical areas of activation for each of the visual conditions, in both the ASD participant and the matched control.
The MRI images demonstrate the highest areas of activation for each visual condition, in the ASD participant and the matched control.

Table 8 and 9 Neutral weighted images comparison: Area, voxel size related to the largest cluster of activation in each anatomical region at p value < 0.001603, Talaraich coordinates of clusters of activation in ASD participant (**table 8**) and age matched control (**table 9**).

Table 8

ASD participant				
Anatomical brain region	**Voxel size**	**x**	**y**	**z**
Left frontal lobe	184	-28	-37	28
Left limbic lobe, cingulate gyrus	88	-18	-6	42
Left parietal lobe, post central gyrus	121	-45	-23	35

Table 9

Control participant				
Anatomical brain region	**Voxel size**	**x**	**y**	**z**
Left frontal lobe - medial frontal gyrus	102 42	-27 -24	35 21	25 42
Right frontal lobe - superior frontal gyrus - pre-central gyrus	242 26	25 39	37 -5	28 34

Figure 2 Highest area of activation in Neutral image condition for ASD participant.
Talairach coordinates x= -28, y= -37, z= 28, Left frontal lobe

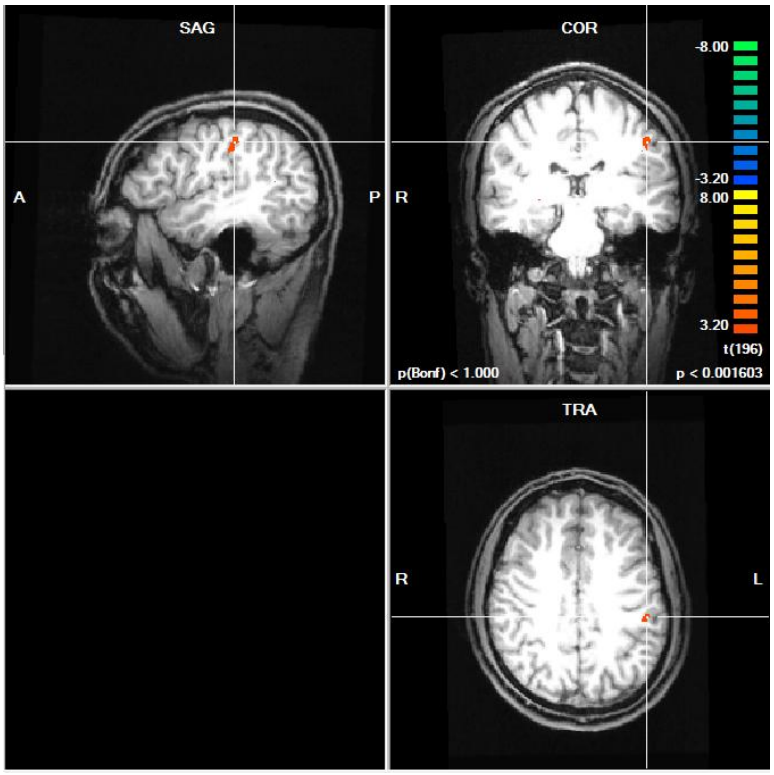


Figure 3 Highest area of activation in Neutral image condition for Control participant.
Talairach coordinates x= -27, y= 35, z= 25, Left frontal lobe

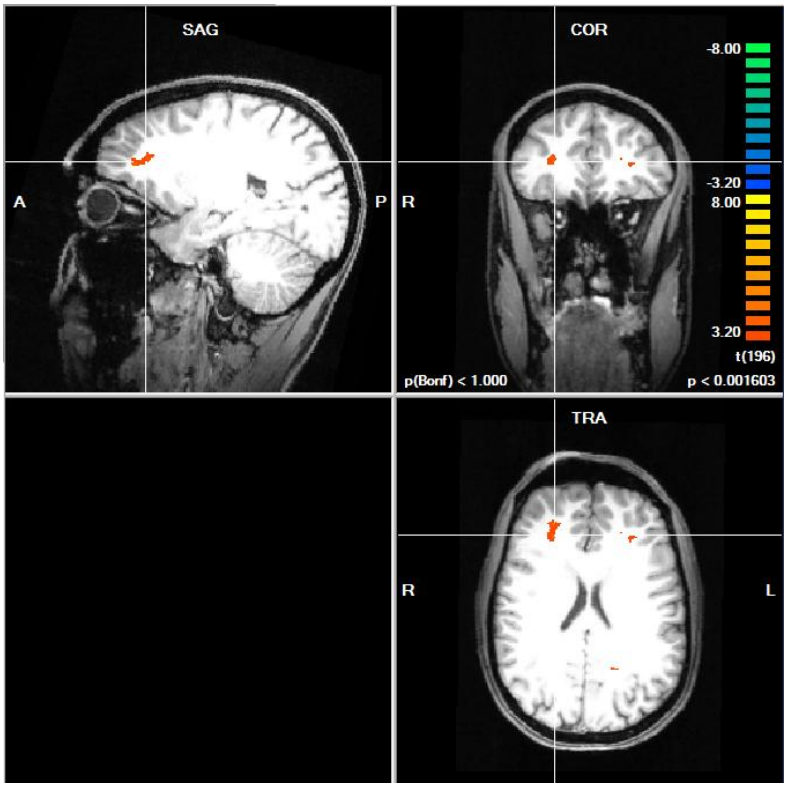


Table 10 and 11 Valance, Arousal and Dominance images comparison: Area, voxel size related to the largest cluster of activation in each anatomical region at p value < 0.001603, Talaraich coordinates of clusters of activation in ASD participant (**table 10**) and age matched control (**table 11**).

Table 10

ASD participant				
Anatomical brain region	**Voxel size**	**x**	**y**	**z**
Left frontal lobe	12	-21	-6	52
Right limbic lobe, cingulate gyrus	118	6	-15	34

Table 11

Control participant				
Anatomical brain region	**Voxel size**	**x**	**y**	**z**
Left frontal lobe	33	-21	17	29
Right temporal lobe	24	34	-56	18
Right parietal lobe	32	22	-58	28

Figure 4 Highest area of activation in Valance, Arousal and Dominance image condition for ASD participant. Talairach coordinates x= 6, y= -15, z= 34, Right limbic lobe.

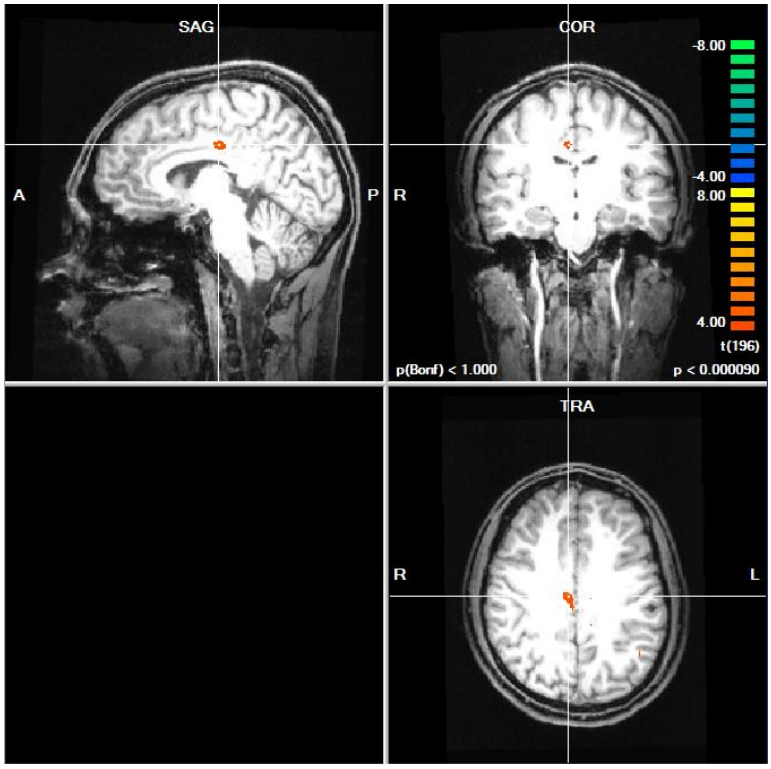


Figure 5 Highest area of activation in Valance, Arousal and Dominance image condition for Control participant. Talairach coordinates x= -21, y= 17, z= 29, Left frontal lobe.

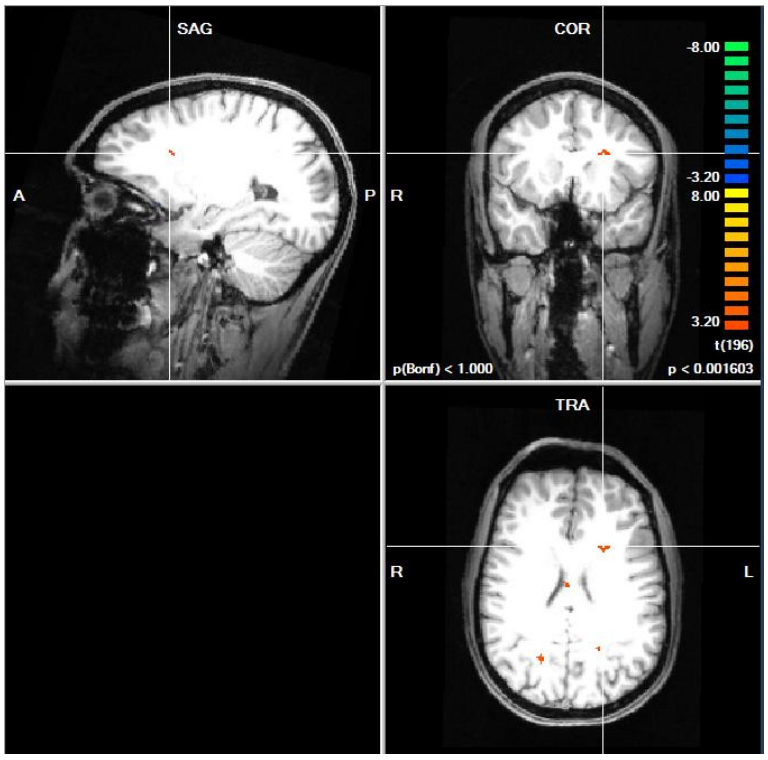


Table 12 and 13 Valance and Dominance images comparison: Area, voxel size related to the largest cluster of activation in each anatomical region at p value < 0.001603, Talaraich coordinates of clusters of activation in ASD participant (**table 12**) and age matched control (**table 13**).

Table 12

ASD participant				
Anatomical brain region	**Voxel size**	**x**	**y**	**z**
Left frontal lobe, superior frontal gyrus	140	-5	34	49
Left temporal lobe, middle temporal gyrus	508	-54	-11	-6
Left limbic lobe, posterior cingulate gyrus	449	-7	-57	17
Left parietal lobe, precuneus	369	-5	-65	24
Right limbic lobe, cingulate gyrus	231	8	-12	30

Table 13

Control participant				
Anatomical brain region	**Voxel size**	**x**	**y**	**z**
Left limbic lobe, cingulate gyrus	21	-16	17	29
Right frontal lobe, cingulate gyrus	40	11	19	34
Right occipital lobe	6	22	-64	28

Figure 6 Highest area of activation in Valance and Dominance image condition for ASD participant. Talairach coordinates x= -54, y= -11, z= -6, Left temporal lobe

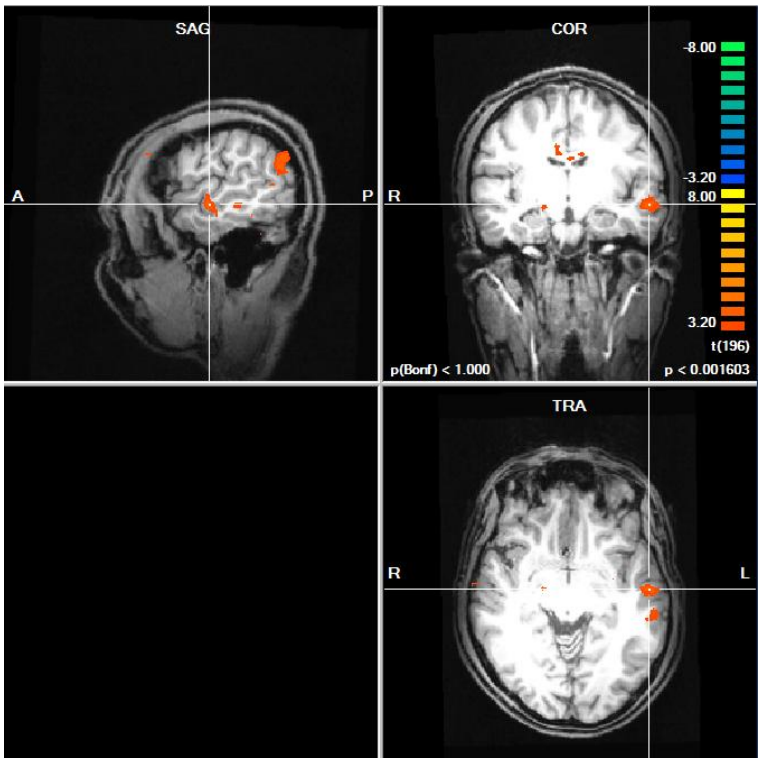


Figure 7 Highest area of activation in Valance and Dominance image condition for Control participant. Talairach coordinates x= 11, y= 19, z= 34, Right frontal lobe, cingulate gyrus

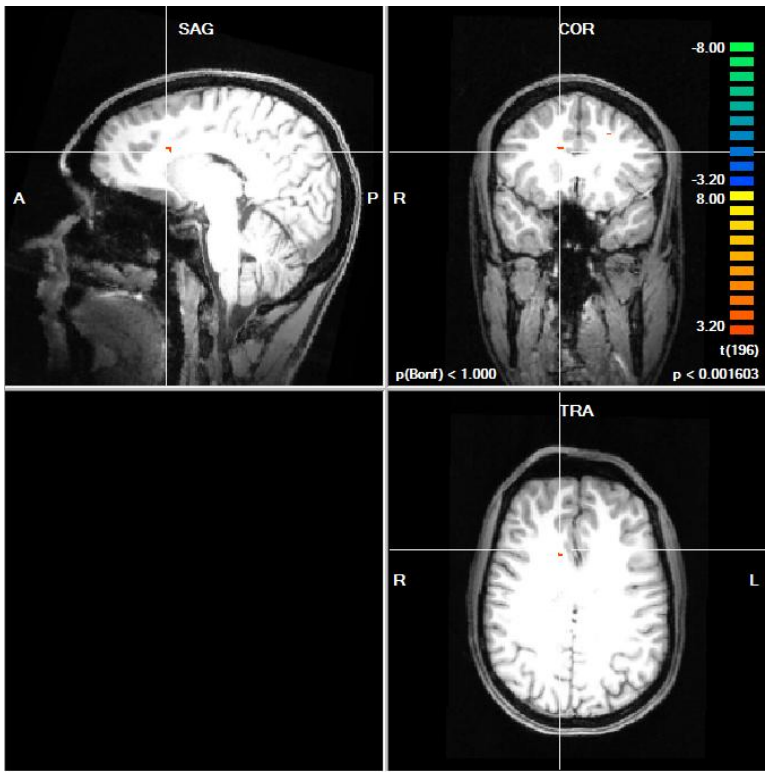


Table 14 and 15 Valance and Arousal images comparison: Area, voxel size related to the largest cluster of activation in each anatomical region at p value < 0.001603, Talaraich coordinates of clusters of activation in ASD participant (**table 14**) and age matched control (**table 15**).

Table 14

ASD participant				
Anatomical brain region	**Voxel size**	**x**	**y**	**z**
Left frontal lobe, medial frontal gyrus	301	-28	-36	28
Left temporal lobe, medial temporal gyrus	85	-49	-18	-13
Left parietal lobe, post central gyrus	132	-42	-27	34
Left limbic lobe, cingulate gyrus	200	-14	-6	37
Right frontal lobe - post central gyrus	215 162	21 40	-24 -19	47 26
Right parietal lobe, precuneus	59	17	-50	30
Right limbic lobe - cingulate gyrus - para-hippocampal	103 35	7 -15	-14 -19	33 54

Table 15

Control participant				
Anatomical brain region	**Voxel size**	**x**	**y**	**z**
Left frontal lobe	123	-25	36	25
Left temporal lobe	34	-25	-62	16
Left parietal lobe	61	-28	-41	24
Right frontal lobe	363	21	29	29
Right parietal lobe, angular gyrus	48	40	-65	32

Figure 8 Highest area of activation in Valance & Arousal image condition for ASD participant. Talairach coordinates x= -28, y= -36, z= 28, Left frontal lobe, ,medial frontal gyrus.

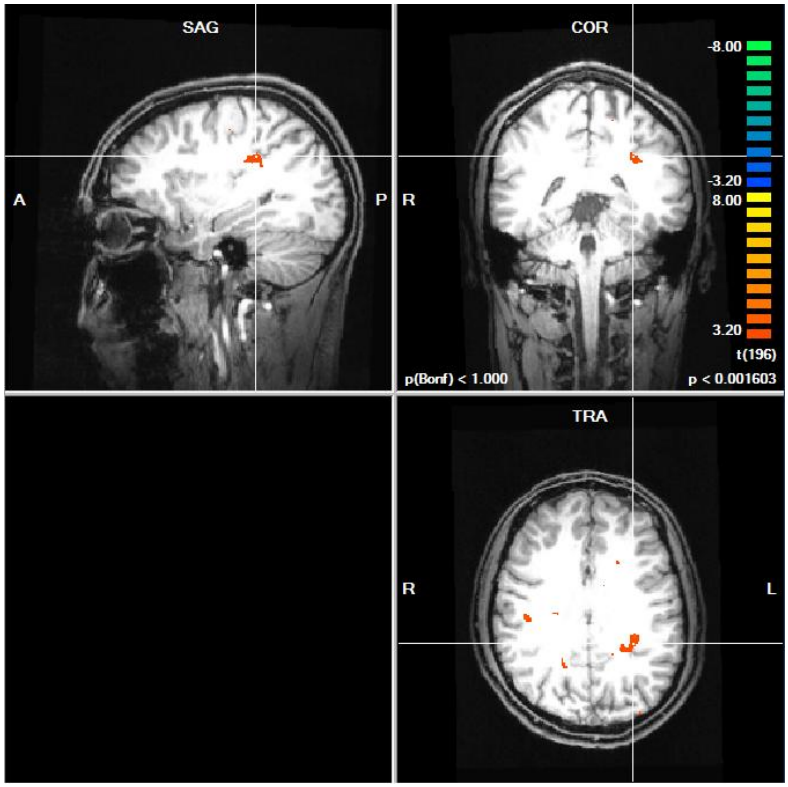


Figure 9 Highest area of activation in Valance & Arousal image condition for Control participant. Talairach coordinates x= 21, y= 29, z= 29, Right frontal lobe

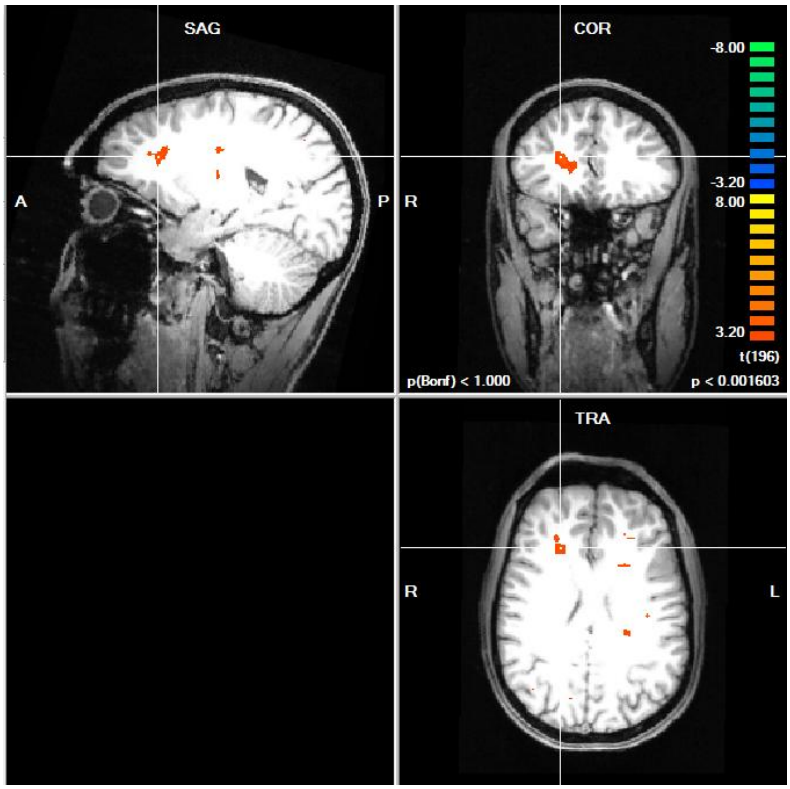


Table 16 and 17 Arousal images comparison: Area, voxel size related to the largest cluster of activation in each anatomical region at p value < 0.001603, Talaraich coordinates of clusters of activation in ASD participant (**table 16**) and age matched control (**table 17**).

Table 16

ASD participant				
Anatomical brain region	**Voxel size**	**x**	**y**	**z**
Left limbic lobe, cingulate gyrus	33	-9	-23	31
Left parietal lobe, precuneus	102	-16	-67	35
Right limbic lobe, cingulate gyrus	41	8	-7	33

Table 17

Control participant				
Anatomical brain region	**Voxel size**	**x**	**y**	**z**
Left limbic lobe	38	-15	-50	28
Left parietal lobe, precuneus	25	-14	-42	43
Right frontal lobe, cingulate gyrus	17	15	21	34
Right temporal lobe, middle temporal gyrus	47	37	-56	21

Figure 10 Highest area of activation in Arousal image condition for ASD participant. Talairach coordinates x= -16, y= -67, z= 35, Left parietal lobe.

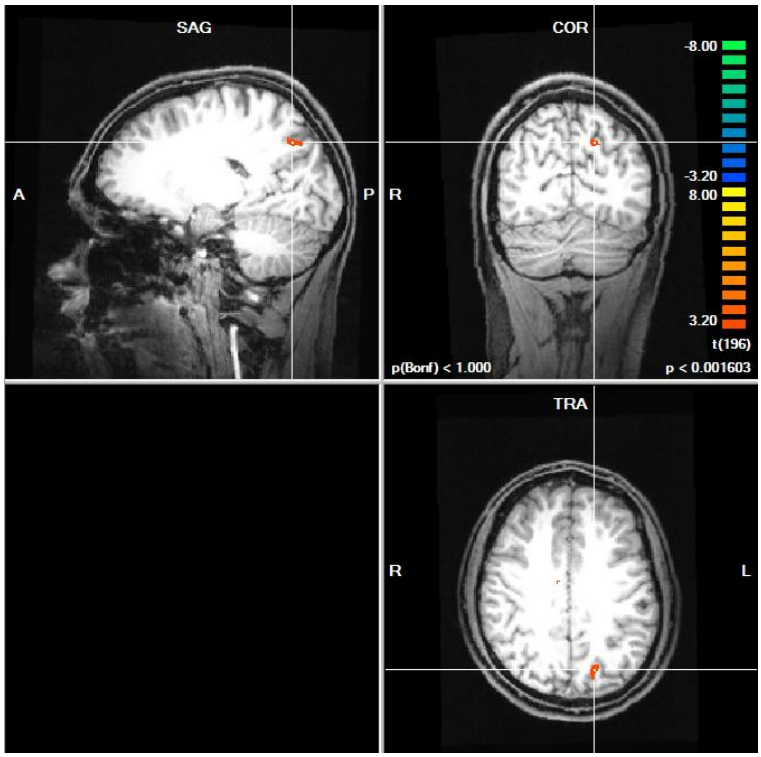
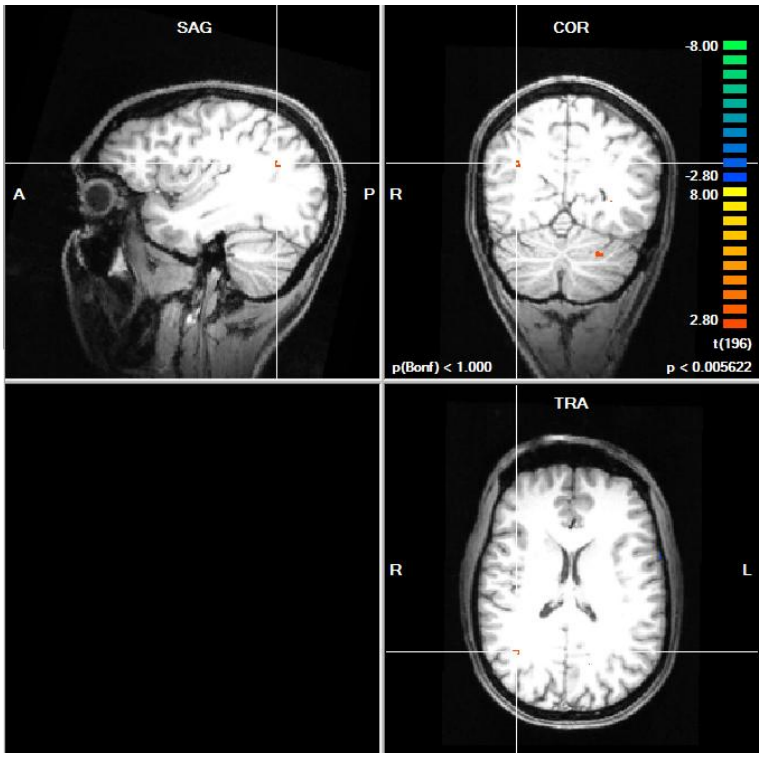


Figure 11 Highest area of activation in Arousal image condition for Control participant. Talairach coordinates x= -37, y= -56, z= 21, Right temporal lobe



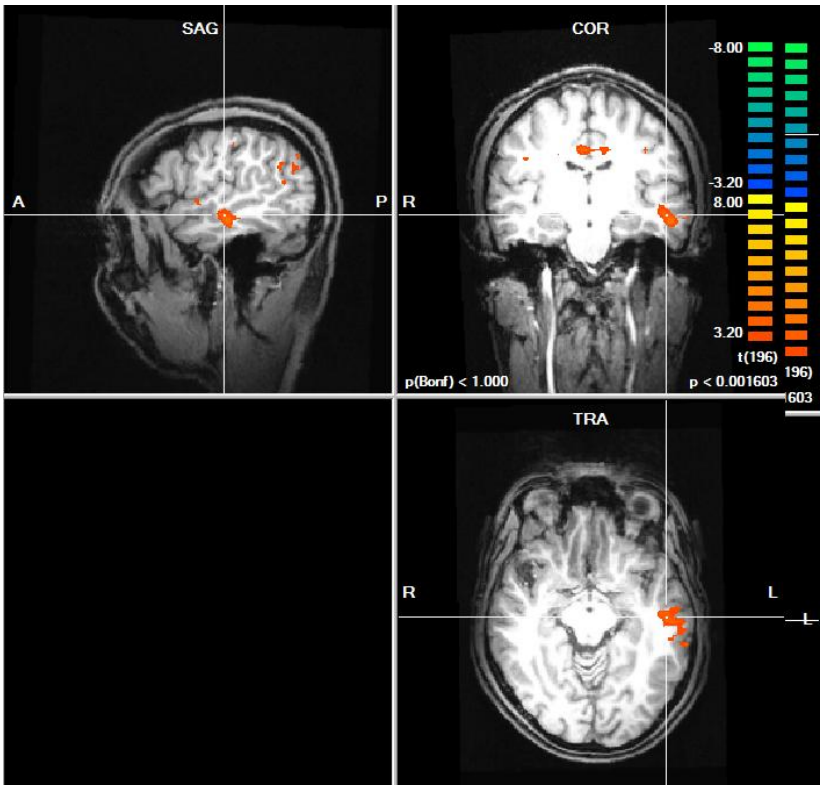
ASD Circumscribed Interests Results

A component of the visual paradigm involved exchanging 15 of the neutral images for 15 visual displays of the ASD participant's circumscribed interests, in order to discover which brain regions were activated from images that were visually rewarding to the participant. Below is a table of the areas of brain activation during the visual paradigm condition involving the ASD participant's circumscribed interests and MRI images demonstrating the highest area of activation in this condition.

Table 18 Circumscribed interest images for ASD participant: Area, voxel size related to the largest cluster of activation in each anatomical region at p value < 0.001603, Talaraich coordinates of clusters of activation in ASD participant.

Anatomical brain region	**Voxel size**	**Coordinate x**	**Coordinate y**	**Coordinate z**
Right frontal lobe, superior frontal gyrus	59	13	18	52
Right limbic lobe, anterior cingulate gyrus	204	6	-15	34
Left frontal lobe - superior frontal lobe - middle frontal lobe	 77 45	 -13 -39	 21 -1	 53 47
Left limbic lobe - anterior cingulate gyrus - parahippocampal gyrus	 350 25	 -8 -24	 -22 -27	 32 -12
Left temporal lobe	694	-50	-16	-9
Left parietal lobe, post central gyrus	276	-42	-26	36

Figure 12 Highest area of activation in Circumscribed interest image condition for ASD participant. Talairach coordinates x= -50, y= -16, z= -9, Left temporal lobe



The actual areas activated by the ASD participant are compared to the predicted areas of brain activation for each of the groups of images in Appendix D.

The main objective for this study was to develop paradigms which would express reward by means of different stimuli. The paradigms produced and then used in the study were made up of visual reward, touch reward and gambling/monetary reward to be tested. The analysis of the visual paradigm results enabled the identification of the differences between the various rewarding and non-rewarding images and the brain areas they activated in normal participants. Following this, the analysis comparing the results of the case and comparing them to one control participant, revealed a great difference between the areas of brain activation of the case and the control. This proof of concept identifies that a difference exists between the ASD participant and the control in the areas of the brain used to process rewarding information. This therefore allows for a progression of the study to a larger scale study which can identify whether the differences observed in this pilot study can be generalised to a population.

Control group: common areas of activation between conditions

All visual stimuli The medial prefrontal cortex, which is one of the most activated regions in all of the conditions is thought to be engaged in emotional self awareness and remembering the feelings of one's own past experiences (202). Medial prefrontal activation is thought to reflect personal past associations and experiences (172). Therefore, it is assumed that these emotional processes were taking place during all of the conditions in the visual task.

All of the image conditions generated activity in the limbic lobe and anterior cingulate gyrus. However, the condition which produced the greatest voxel size of activity was the high valance and high arousal condition. It has been suggested that high valance and arousal IAPS images require increased cognitive demands in order to process the high emotional content of the images (172). With the anterior cingulate gyrus playing a role in evaluating personal experiences from visual stimuli and processing reward anticipation and emotion, a suggestion can be made that the anterior cingulate gyrus is activated in response to pleasure and reward orientated stimuli (172,203,204).

The fusiform gyrus is believed to have a role in appraising both positive (pleasurable) and negative (fearful and sad) stimuli, subsequently the results from

this study demonstrating activation in the fusiform gyrus during high valance and dominance images correlates with previous findings. However, the other image conditions did not produce any activation in the fusiform gyrus, including the high arousal condition which contained fear and sadness inducing images. It is unclear as to why the fusiform gyrus was not activated from the high arousal images as a past study which investigated the regions of the brain activated in response to faces of different emotional expressions, found that fear inducing images produced high activation in the fusiform gyrus.

Neutral The medial frontal gyrus, the anterior cingulate gyrus and the middle temporal gyrus were activated by the neutral image condition. The medial frontal gyrus occupies part of the anterior prefrontal cortex which functions to organise and understand complex tasks, whilst the middle temporal gyrus is connected with processes such as recognition of faces and images (205,206). The participants had never previously seen the images displayed to them during the scan; therefore the findings suggest that these areas are involved in recognising objects, places and faces in the neutral images. The anterior cingulate gyrus has connections with the amygdala, nucleus accumbens, hypothalamus and anterior insula and functions to assess emotional and motivational behaviour (207). Whilst these images contained neutral emotional weightings, the participants were aware before the scan that images of some emotional value would be displayed to them whilst they were in the MRI scanner. Various images classified as neutral may have been rewarding to particular individuals, consequently the anterior cingulate gyrus could be involved due to certain images that could have emotional value to the participants who took part in the study. The implications that this could have on the findings of this study is that if some images that were deemed to have neutral emotional ratings in the IAPS library actually have an emotional impact on some participants, this may affect the comparison of brain activation between the neutral images and the images rated as having some kind of emotional value.

Valance, Arousal & Dominance The superior frontal gyrus, the medial frontal gyrus, the anterior cingulate gyrus, the precuneus and the supramarginal gyrus were activated by the high valance, high arousal and high dominance image condition. The superior frontal and medial frontal gyri are both part of the prefrontal cortex, an area

of the brain that deals with executive functions such as differentiating between thoughts, balancing and predicting the consequences of actions and goal orientated behaviour (208). It is also thought that the prefrontal cortex, particularly the medial area, is involved in processing emotions and relating oneself to the emotions seen in visual stimuli. These considerations together raise the notion that the high activation in different areas of the prefrontal cortex could be due to the high emotional content of the images shown during the condition (such as erotic scenes and action sports) which demand increased cognitive input to process the highly emotional images. The supramarginal gyrus has a role in receiving auditory, visual and somatosensory stimuli (209), therefore it could have a function in receiving and processing the visual stimuli in the paradigm. The anterior cingulate gyrus and the limbic lobe, which consists of the amygdala, insula and thalamus, both have a role in reward anticipation and in the management of emotion (203,204). Consequently it can be assumed that their increased activation during this particular condition of the paradigm is due to their roles in processing emotional interactions and emotional awareness, which corresponds with work of previous studies (210,211).

Valance & Dominance The right and left frontal lobes, anterior cingulate gyrus, the limbic lobe and the fusiform gyrus were activated by the high valance and high dominance images. The images displayed in this condition of the visual paradigm contained high pleasure and high control emotional content, such as images of cute animals and babies. Past studies have reported that sadness rather than happiness in stimuli activate the anterior cingulate gyrus (212,213). This present study investigated the neural response to emotional images by men, however it is important to note that the IAPS image scores were based on both male and female ratings, therefore what males may deem as emotionally pleasurable may be different to males and females combined. Therefore the anterior cingulated gyrus activation in this condition reveal that the male controls in this study did not find the high valance and high dominance images pleasurably rewarding. The activation demonstrated in this condition was not as high as the activation exhibited in other regions of the brain such as the frontal lobe. The orbitofrontal cortex, an anterior area of the frontal lobe, has been associated with appraising positive social and emotional stimuli, which could be a link as to why the frontal lobes, particularly the right frontal lobe, were highly activated during this condition of the task (136,214). The fusiform gyrus is

believed to have a role in face recognition and has also been linked to the processing of both fear inducing and pleasurable visual stimuli (215,216). This information agrees with the finding of fusiform gyrus activation from high valance and arousal images in this study.

Valance & Arousal The medial frontal gyrus, the precentral gyrus, the anterior cingulate gyrus, the precuneus and the insula were activated by the high valance and high arousal image condition. The images in this condition contained emotions such as excitement, joy and pleasure, as well as demonstrating static situations of movement and actions. The high activation in the anterior cingulate gyrus is therefore consistent with previous findings that social emotions, such as joy, activate areas around the amygdala and the cingulate cortex (172,217). However, in a recent study which used IAPS images to examine the neural activation from images of expressive faces in comparison IAPS images, the positive (joyful, pleasurable) IAPS images failed to activate the amygdala (172). The precentral gyrus lies within the primary motor cortex which plans and executes movements (218). It has been found that neurons in the precentral gyrus of monkeys respond to visual and tactile stimuli (219). This could correlate with the pre-central gyrus activation in this study from visual stimuli, however no previous fMRI studies in humans have found pre-central gyrus activation due to visual stimuli. Neuro-imaging studies have found that the precuneus, located in the posterior area of the parietal lobe, has a role in visuo-spatial imagery and memory retrieval (220). In past studies, visual-verbal stimuli have activated the precuneus (221). Past studies have observed an increased activation in the precuneus during imagined movement, more than actual movement (222,223). For that reason, a suggestion can be made that the activation of the precuneus during the visualisation of high valance and high arousal images could be due to the active and movement nature of the images, indicating that the precuneus could be involved in producing the spatial information required for imagined body movements, which could be what participants are imagining whilst seeing the images in this condition. The insula plays a role in perception, motor control and the experience of emotions, which correlates with a past study that not only suggested that IAPS images with a high valance and arousal require increased cognitive demands in order to process, but also that the insula may have a role in assessing more complex stimuli (172).

Arousal The frontal lobe, the medial frontal gyrus and the anterior cingulate gyrus were activated by the high arousal images. The nature of images displayed in this condition were that of negative, sad, fear inducing images, such as mutilation, gangs and starving children. The anterior cingulate gyrus has been found to show activation from stimuli demonstrating negative emotions (172,212). The anterior cingulate gyrus has connections with the amygdala and past studies have demonstrated the amygdala's involvement and activation during the processing of negative emotions (213). Past research using IAPS images to assess gender differences in processing emotional visual stimuli discovered that women showed greater brain activity in the anterior and medial cingulate gyri from affectively negative images (224). This leads to the impression that the anterior cingulate gyrus plays a role in dealing with negative visual stimuli such as those displayed in the high arousal condition in this study. In this present study only males were scanned, therefore future work can look at the differences in activation during the paradigms of this study between males and females. The medial frontal gyrus was activated during this condition, which correlates with findings in a previous study stating that IAPS images of a negative content activated the medial prefrontal cortex, illustrating that it may a have a role in assessing and processing negative emotion (172). However, results from a different study observed high activation in the inferior frontal gyrus from negative emotional images, which was not activated in this group of participants (224). The frontal gyrus activation could be investigated in more depth in future fMRI work using the paradigms used in this study.

Case vs. Control comparison

The ASD participant who was scanned in this study was compared to an aged matched participant from the control group in order to consider the similarities and differences in the neural regions activated during the visual paradigm. The significant differences in activation between the case and control cannot be generalised to a population due to the small number of participants. In the analysis the p-value for the comparison of the case and the control was set at < 0.001603. This p-value is higher than that set for the control group batch analysis (set at < 0.000012) as the activation in each individual participant's fMRI data was less than

that in the control group batch analysis, therefore this p value was chosen to demonstrate significant activation at the lowest p-value.

In all of the conditions of the visual paradigm it was evident that the ASD participant demonstrated more general neural activation than the control participant. The limbic regions and the anterior cingulate gyrus were activated during all of the conditions in the ASD participant, whilst the limbic lobe was activated in just the high arousal condition and the high valance and dominance condition in the control participant. This increased activation during all of the conditions in the ASD participant could be explained by a theory that individuals with ASD have difficulties in modulating their arousal state, in that they change between the two extremes of under and over arousal (225). Past studies have argued that children with ASD have chronic high levels of arousal, which consequently makes them intolerant to sudden changes in arousing stimuli (226). Therefore, if individuals with ASD are exposed to a constant stream of arousing stimuli, their neural response may over respond to the stimuli no matter what the arousal or valance levels that each stimulus carries. This evidence may have implications on the reward processing ability of people with ASD. As they struggle to distinguish between rewarding and non rewarding stimuli, assessments of activation differences between ASD participants and controls may be difficult due to factors such as over-arousal in people with ASD.

An interesting finding was discovered in the ASD participant's fMRI results which revealed activation in the parahippocampal gyrus during the high valance and arousal condition and also in the circumscribed interest condition. The parahippocampal gyrus is a region of the brain that surrounds the hippocampus and has been found to play an important role in visual memory retrieval and the recognition of scenes rather than faces (227-229). The high valance and arousal condition contained scenes such as high action sports and landscapes which lead to an assumption that the parahippocampal gyrus was activated in the ASD participant whilst he viewed this condition due to the nature of the images. However, the individual control participant and the control group as a whole demonstrated no activation in the parahippocampal gyrus during any of the conditions of the visual paradigm. The circumscribed interest condition was solely displayed to the ASD participant in exchange for fifteen of the neutral images. These images consisted of 3 interests (Animaniacs, Family guy, Sonic the hedgehog), therefore 5 images per interest were displayed during the visual task. During this condition the

parahippocampal gyrus was activated. This could be explained by the previous theory that this region of the brain is involved in memory retrieval along with recognition of scenes, which could also include scenes such as those from television programmes and games, like the circumscribed interest images used for this participant.

The development of the four paradigms allowed for reward to be assessed in various ways. The visual paradigm demonstrates reward in positive emotion and non reward in negative emotional stimuli. The results for the brain activation in the control participants display a difference between not only the rewarding and non rewarding stimuli, but also between the different types of rewarding stimuli. These differences allow us to appreciate the variation in the types of reward that can be produced. By becoming aware of the different areas of brain activation in control participants we can then compare these areas with the brain activation demonstrated by ASD participants. The results for the ASD participant from this study revealed increased areas of activation. This information can be put forward in order to carry out a larger study which will allow for comparisons of not only rewarding and non rewarding visual emotional stimuli, but also of the different types of reward which can be tested with the further paradigms.

Limitations

Participants - The key limitation from this project was the small number of participants in both the control and the ASD group. The initial control group number was 11 participants, however various circumstances arose which lowered the control group to 7 participants who were included in the final analysis.

The recruitment of ASD participants for the case group was more difficult than initially expected. Although the support groups mentioned in the results-recruitment section were visited and adverts were put into newsletters each of their newsletters and on the University intranet, there was a lack in people showing interest in participating. The difficulty in recruiting participants for the ASD group could have been due to a number of reasons such as the social difficulties that people with ASD suffer from playing a role in their decision of participating or not. Many carers and parents of individuals with ASD were very interested in their sons taking part in the study however they had trouble in convincing the individuals in participating. This

lack of participants prompted the decision for the participant age group to be increased from 18-25 years to 18-40 years. This was approved by the local ethics committee. This information was then relayed back to the support groups who re-advertised for participants and a revised advert was placed on the University intranet site. The difficulties met in this recruitment process have taught us that with the type of individuals we were trying to recruit, the inclusion criteria must be developed in order to support more people to volunteer as participants. In the next phase of the larger study it is possible that the inclusion criteria will have to include a larger age range from the outset, a lower IQ range and female participants will have to be considered.

IQ test – An additional IQ test can be used along with the current test, The National Adult Reading Test, in order to improve the testing process to ensure that the test is specific and identifies a specific IQ, without the participants having to endure a long IQ tests.

Favourite images - Favoured images were chosen for the ASD participants to include in the visual task in order to observe which brain regions are activated from the participant's interests (rewarding to the participant). This should be carried out for the control participants in order to compare the brain activation from favoured images for each participant. A group comparison for this particular group of images can then be carried out in order to compare the controls to the ASD participants.

Analysis – The paradigms have to be analysed separately and each individual paradigm would take many steps to analyse and produce activation that can be localised to a particular anatomical and then functional brain region. Therefore, to analyse the visual paradigm was the main focus for this study.

Statistics – The small number of participants in both groups but especially in the ASD group meant that it was not possible to carry out a statistical analysis to compare the significant activation between the control group and the ASD group. Although similarities with previous research results were found in this small participant data set, theories to explain the regions of activation in the results found

can only be suggested. Further work into this study could produce stronger conclusions into the neural activation during these paradigms.

Further work and Conclusions

This study, which is primarily analysing the visual aspect of reward in normal participants, is a preliminary study, which focuses on a segment of a larger study regarding the effect of different forms of reward on brain activation in normal and ASD participants. The results from this study not only demonstrate a difference between the various groups of images in the visual paradigm and the brain areas that they activate, the results also display a clear difference between the brain activation of the ASD participant and the control participants. This information allows for the assumption that in a larger study, which will compare the results of a larger number of participants, will therefore reveal more significant differences between the brain activation in control participants and ASD participants from rewarding tasks.

Further work should be carried out to recruit additional participants for both the control and the ASD participant group in order to complete the study and carry out statistical analyses to compare the regions of activation between the groups. As the touch and the gambling paradigms were not analysed here, the recruitment of more participants will allow for the data from these paradigms to be analysed and statistically verified. Only suggestions could be made regarding the reasons for particular regions to show activation as the number of participants did not allow for statistically significant links in activation patterns. With the continuation of work on this project, further data will allow for more significant conclusions to be formed regarding the neural activation patterns in both the control group and the ASD group. It is worthwhile carrying out the tasks used in this study in a further fMRI work in individuals with ASD, as the evidence has already been found regarding the problems with reward processing in people with ASD. This study, although only demonstrating data from one ASD participant, displays a clear difference between our ASD participant

and controls. It will be possible to find out whether these differences in activation are indeed due to differences in the neural reward processing of ASD participants in comparison to controls, with future fMRI research that recruits more ASD participants. This data will move forward thinking regarding the reward processing of different types of reward tests in people with and without ASD.

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Appendix A - Tables of images from IAPS database

Table a1 Neutral Images

Image	Image number	Picture set	Valence mean	Arousal mean	Dominance mean
Snake	1112	8	4.83	4.40	5.00
Spider	1230	1	4.25	4.98	4.92
Rat	1280	4	4.40	4.48	5.12
Woman	2130	2	4.20	4.90	5.29
Sadface	2230	3	4.67	4.12	5.04
Mother	2312	12	4.00	3.77	5.04
Elderly Man	2520	3	4.12	4.19	4.65
Cowboy	2635	14	5.26	4.45	5.34
Bomb	2692	10	4.02	5.11	4.82
Police	2694	13	4.18	4.93	5.11
Refugees	2695	13	4.49	4.26	4.71
Actor	2780	13	4.75	4.70	5.25
Boy	2795	14	4.09	4.37	5.15
Scar	3190	4	4.21	5.17	4.52
Surgery	3210	2	4.83	5.27	4.49
Prostitute	4635	10	4.60	4.05	5.56
Tornado	5970	4	4.31	4.65	3.79
Prison	6000	3	4.19	4.83	4.06
Electricchair	6020	4	4.10	5.23	4.65
Aimedgun	6190	4	4.52	4.83	4.54
Lonely boy	2272	13	4.50	3.74	5.24
Braces	2279	18	4.71	3.74	5.55
Girlcow	2309	18	4.89	4.33	5.39
Woman	2372	11	5.48	4.09	5.72
Secretary	2383	12	4.72	3.41	5.75
Couple	2396	16	4.91	3.34	5.59
Boy	2410	20	4.62	4.13	5.00
Neutral girl	2441	13	4.64	3.62	5.57
Boots	2446	15	4.70	3.79	5.51
Crying baby	2458	20	4.69	5.28	5.06
Man	2490	5	3.32	3.95	4.72
Police 2	2681	10	4.04	4.97	3.84
Police 3	2682	9	3.69	4.48	4.02
Terrorist	2690	4	4.78	4.02	4.91
Woman	2700	4	3.19	4.77	4.44
Soldiers	2704	15	4.85	5.30	4.86
Smoking	2715	13	3.28	4.35	5.17
Pipe	2716	16	3.54	4.97	4.7
Mask	2770	15	4.37	5.11	4.82
Coach	3550.2	11	4.92	5.13	5.38
Snake	1080	1	4.24	5.69	4.33
Dog	1303	11	4.68	5.70	4.98
Bees	1390	3	4.50	5.29	4.75
Dograce	1505	17	4.13	4.73	4.49
Wolf	1645	18	4.99	5.14	4.74
Crocodile	1820	19	5.35	5.67	4.66
Jellyfish	1908	17	5.28	4.88	4.75
Hermit crab	1935	14	4.88	4.29	5.5

Turtle	1945	11	4.59	4.42	5.57
Woman 2	2026	17	4.82	3.40	5.09
Angry face	2100	1	3.85	4.53	5.05
NeuWoman	2104	15	4.42	3.11	5.45
Angry face	2110	1	3.71	4.53	4.66
Body Pierce	2115	19	3.83	4.98	4.87
Angry face	2120	1	3.34	5.18	4.52
Neut face	2200	1	4.79	3.18	5.44
Fingerprint	2206	10	406	3.71	4.46
Neut face	2210	1	4.38	3.56	5.23
Man	2211	17	5.19	4.05	5.25
Judge	2221	9	4.39	3.07	4.97

Table a2 High Valance, High Arousal, Low Dominance

Image	Image Number	Picture Set	Valance mean	Arousal mean	Dominance mean
Waterfall	5260	6	7.47	6.00	4.18
Astronaut	5470	6	7.38	6.44	4.75
Mountains	5600	2	7.27	5.93	4.78
Sky	5982	6	7.38	5.25	4.90
Cliffdivers	8180	4	7.50	6.54	4.83
Skier	8030	2	7.33	7.35	4.70
Parachute	8163	20	7.14	6.53	5.69
Sailboat	8170	6	7.63	6.12	5.72
Cliffdiver	8178	13	6.50	6.82	4.68
Bungee	8179	13	6.48	6.99	4.73
Ice Climber	8191	14	6.07	6.19	4.88
Wing walker	8341	13	6.52	6.40	4.66
Rafters	8400	6	7.09	6.61	4.63
Rollercoaster	8490	4	7.20	6.68	5.37
Rollercoaster	8492	17	7.21	7.31	4.63

Table a3 High Valance, High Arousal, High Dominance

Image	Image Number	Picture Set	Valance mean	Arousal mean	Dominance mean
Skier	8190	5	8.13	6.41	6.17
Attractivefem	4250	6	8.39	7.02	6.06
Eroticfemale	4220	1	8.25	7.80	6.97
Eroticfemale	4180	1	8.21	7.43	6.41
Puppies	1710	3	8.02	5.53	6.61
Erotic couple	4608	7	7.07	6.47	6.25
Erotic couple	4680	4	7.25	6.02	6.27
Skier	8034	7	7.06	6.30	6.26
Waterskier	8200	3	7.54	6.35	6.17
Money	8501	6	7.91	6.44	6.05
Erotic couple	4652	8	6.79	6.62	6.10
City	7650	20	6.62	6.15	5.79
Sailing	8080	2	7.73	6.65	5.91
Gymnast	8470	6	7.74	6.14	6.17
Waterslide	8496	9	7.58	5.79	6.33

Table a4 Low Valance, High Arousal, Low Dominance

Image	Image Number	Picture Set	Valance mean	Arousal mean	Dominance mean
War	2683	13	2.62	6.21	3.43
Hunters	2688	14	2.73	5.98	3.99
Sad children	2703	16	1.91	5.78	3.15
Mutilation	3000	1	1.45	7.26	2.99
Accident	3015	11	1.52	5.90	2.84
Attack	6560	5	2.57	6.17	3.87
Gang	6821	10	2.96	5.93	3.95
HIVTattoo	9006	7	2.63	5.29	3.97
Starvingchild	9040	2	1.88	5.10	3.27
Dog	9570	6	1.90	5.84	3.49
Attack dog	1525	13	3.09	6.51	3.15
Toddler	2095	14	1.79	5.25	3.7
Grieving fem	2141	11	2.44	5.00	3.92
Black eye	2345.1	17	2.26	5.50	3.96
Bloody kiss	2352.2	12	2.09	6.25	3.45

Table a5 High Valance, Low Arousal, High Dominance

Image	Image Number	Picture Set	Valance mean	Arousal mean	Dominance mean
Cat	1540	1	7.15	4.54	7.01
Butterfly	1604	9	7.11	3.30	6.69
Antelope	1620	1	7.37	3.54	6.82
Fawn	1630	20	7.26	4.45	6.12
Kid	2035	18	7.52	3.69	6.20
Women	1340	11	7.13	4.75	6.13
Ferret	1410	18	7.00	4.17	6.05
Gannet	1450	1	6.37	2.83	6.75
Kitten	1460	5	8.21	4.31	6.00
Dog	1500	1	7.24	4.12	6.97
Seal	1440	6	7.96	4.76	6.33
Polarbears	1441	15	7.71	3.84	6.71
Kittens	1463	8	7.10	4.46	6.33
Rabbit	1610	2	7.32	4.24	6.49
Porpoise	1920	4	7.83	4.21	6.42

Appendix B sequence of exposure of images, fixation cross and their timings

Illustration on the screen	Times
The visual experiment is about to begin	3500
fix cross	2500
police 1	1000
fix cross	4000
cliffdiver	1000
fix cross	4000
neuwoman	1000
fix cross	4500
butterfly	1000
fix cross	3500
aimedgun	1000
fix cross	4500
gang	1000
fix cross	4000
mask	1000
fix cross	3500
eroticfemale1	1000
fix cross	4000
turtle	1000
fix cross	4500
iceclimber	1000
fix cross	4500
elderlyman	1000
fix cross	3500
ferret	1000
fix cross	4000
man2	1000
fix cross	3500
war	1000
fix cross	4500
dog2	1000
fix cross	4000
eroticcouple1	1000
fix cross	3500
smoking	1000
fix cross	3500
rollercoaster1	1000
fix cross	4500
soldiers	1000
fix cross	4000
polarbears	1000
fix cross	4000
man1	1000
fix cross	4500
attackdog	1000
fix cross	3500
prostitute	1000
fix cross	4500
city	1000

fix cross	4000
electricchair	1000
fix cross	4500
astronaut	1000
fix cross	3500
neutface1	1000
fix cross	4000
dog1	1000
fix cross	3500
boy1	1000
fix cross	4000
dog3	1000
fix cross	4500
angryface1	1000
fix cross	4500
money	1000
fix cross	3500
actor	1000
fix cross	3500
rollercoaster2	1000
fix cross	4000
jellyfish	1000
fix cross	4500
women	1000
fix cross	4500
angryface2	1000
fix cross	4000
attack	1000
fix cross	3500
woman1	1000
fix cross	4000
sailing	1000
fix cross	4000
fix cross	2500
prison	1000
fix cross	4000
cliffdivers	1000
fix cross	4000
woman2	1000
fix cross	4500
seal	1000
fix cross	3500
refugees	1000
fix cross	4500
blackeye	1000
fix cross	4000
police 2	1000
fix cross	3500
eroticcouple2	1000
fix cross	4000
woman3	1000
fix cross	4500
skier1	1000
fix cross	4500

cowboy	1000
fix cross	3500
rabbit	1000
fix cross	4000
angryface3	1000
fix cross	3500
starvingchild	1000
fix cross	4500
neutralgirl	1000
fix cross	4000
eroticfemale2	1000
fix cross	3500
terrorist	1000
fix cross	3500
parachute	1000
fix cross	4500
girlcow	1000
fix cross	4000
gannet	1000
fix cross	4000
fingerprint	1000
fix cross	4500
hunters	1000
fix cross	3500
judge	1000
fix cross	4500
waterskier	1000
fix cross	4000
tornado	1000
fix cross	4500
waterfall	1000
fix cross	3500
bodypierce	1000
fix cross	4000
antelope	1000
fix cross	3500
police3	1000
fix cross	4000
sadchildren	1000
fix cross	4500
neutface2	1000
fix cross	4500
attractivefem	1000
fix cross	3500
pipe	1000
fix cross	3500
sailboat	1000
fix cross	4000
coach	1000
fix cross	4500
kitten	1000
fix cross	4500
scar	1000
fix cross	4000

mutilation	1000
fix cross	3500
surgery	1000
fix cross	4000
waterslide	1000
fix cross	4000
fix cross	2500
snake1	1000
fix cross	4000
bungee	1000
fix cross	4000
crocodile	1000
fix cross	4500
cat	1000
fix cross	3500
lonelyboy	1000
fix cross	4500
toddler	1000
fix cross	4000
boots	1000
fix cross	3500
skier2	1000
fix cross	4000
couple	1000
fix cross	4500
rafters	1000
fix cross	4500
woman4	1000
fix cross	3500
fawn	1000
fix cross	4000
cryingbaby	1000
fix cross	3500
grievingfem	1000
fix cross	4500
sadface	1000
fix cross	4000
eroticcouple3	1000
fix cross	3500
boy2	1000
fix cross	3500
wingwalker	1000
fix cross	4500
bomb	1000
fix cross	4000
kittens	1000
fix cross	4000
dograce	1000
fix cross	4500
bloodykiss	1000
fix cross	3500
hermitcrab	1000
fix cross	4500
skier3	1000

fix cross	4000
mother	1000
fix cross	4500
mountains	1000
fix cross	3500
secretary	1000
fix cross	4000
porpoise	1000
fix cross	3500
wolf	1000
fix cross	4000
hivtattoo	1000
fix cross	4500
bees	1000
fix cross	4500
puppies	1000
fix cross	3500
spider	1000
fix cross	3500
sky	1000
fix cross	4000
rat	1000
fix cross	4500
kid	1000
fix cross	4500
snake2	1000
fix cross	4000
accident	1000
fix cross	3500
braces	1000
fix cross	4000
gymnast	1000
fix cross	4000

Appendix C Actual areas of brain activation in the control group vs. Predicted areas of activation

Actual areas of activation in the control group of this study

Brain Regions	Visual Paradigm Conditions				
	Neutral	*Valance, Arousal & Dominance*	*Valance & Dominance*	*Valance & Arousal*	*Arousal*
Left frontal lobe	209	199	79	342	16
Right frontal lobe	511	132	396	238	
Left temporal lobe	164	170		187	
Right temporal lobe					
Left limbic lobe	181		42		5
Right limbic lobe		122		185	
Left parietal lobe		170		222	
Right parietal lobe				242	
Left occipital lobe	140		15		
Right occipital lobe					
Left fusiform gyrus			15		
Right fusiform gyrus					
Left insula				348	
Right insula					

Predicted areas of activation for a normal control group

Brain Regions	Visual Paradigm Conditions				
	Neutral	*Valance, Arousal & Dominance*	*Valance & Dominance*	*Valance & Arousal*	*Arousal*
Left frontal lobe	✓	✓	✓	✓	✓
Right frontal lobe	✓	✓	✓	✓	✓
Left temporal lobe		✓			
Right temporal lobe		✓			
Left limbic lobe		✓	✓	✓	✓
Right limbic lobe		✓	✓	✓	✓
Left parietal lobe					
Right parietal lobe					
Left occipital lobe	✓	✓			
Right occipital lobe	✓	✓			
Left fusiform gyrus		✓	✓		✓
Right fusiform gyrus		✓	✓		✓
Left insula				✓	
Right insula				✓	

Appendix D Actual areas of brain activation in the ASD participant vs. Predicted areas of activation

Brain Regions	**Visual**		**Paradigm**	**Condition**		
	Neutral	Valance, Arousal & Dominance	Valance & Dominance	Valance & Arousal	Arousal	Circumscribed interests
Left frontal lobe	184	12	140	301		77
Right frontal lobe				215		59
Left temporal lobe			508	85		694
Right temporal lobe						
Left limbic lobe	88		449	200	33	350 anterior cingulate
Right limbic lobe		118	231	103		204
Left parietal lobe	121		369	132	102 precuneus	276
Right parietal lobe				59 precuneus		
Left occipital lobe						
Right occipital lobe						
Left fusiform gyrus						
Right fusiform gyrus						
Left insula						
Right insula						

	Visual		**Paradigm**	**Condition**		
Brain Regions	Neutral	Valance, Arousal & Dominance	Valance & Dominance	Valance & Arousal	Arousal	Circumscribed interests
Left frontal lobe	✓	✓	✓	✓	✓	✓
Right frontal lobe	✓	✓	✓	✓	✓	✓
Left temporal lobe						
Right temporal lobe						
Left limbic lobe		✓ ?	✓ ?	✓ ?	✓ ?	✓
Right limbic lobe		✓ ?	✓ ?	✓ ?	✓ ?	✓
Left parietal lobe						
Right parietal lobe						
Left occipital lobe						
Right occipital lobe						
Left fusiform gyrus						✓
Right fusiform gyrus						✓
Left insula						
Right insula						